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(54) Title: REACTION VESSEL AND SYSTEM INCORPORATING SAME

(57) **Abstract:** A reaction vessel for carrying out at a chemical process and for enabling measurement of parameters correlated to the process, and a system incorporating the vessel. The vessel, which is preferably disposable, has a plurality of upper holding chambers for containing chemicals needed for the process, each holding chamber having a lower outlet and an upper inlet. A plug selectively opens and seals the upper inlet of each chamber. The outlets enable the chemicals to flow into a reaction chamber in a desired sequence for carrying out said process only when the corresponding upper inlet is open. The reaction chamber has a vent with respect to the outside of the vessel. A monitoring electrode is exposed to the reaction chamber for measuring parameters associated with the chemical process. A lower waste chamber having an upper outlet is separated from the reaction chamber via a screen element, and a second plug selectively opens and seals this outlet. The screen member is characterized in selectively preventing and allowing liquid communication therethrough, according to whether the upper outlet is sealed or open, respectively.

REACTION VESSEL AND SYSTEM INCORPORATING SAME

Field of the Invention

The present invention relates to a reaction vessel for performing processes or reactions therein, in particular amperometric immunoassays with high sensitivity and efficiency particularly, but not exclusively, for the determination of thromboxane. The present invention also relates to a system for carrying out amperometric immunoassays, in particular having such a reaction vessel and a suitable means adapted for controlling the operation thereof and for monitoring the electrical response associated with the immunoassay.

Background of the Invention

The group of diseases affecting the heart and blood vessels is one of the leading causes of morbidity and mortality. In the U.S. for example, cardiovascular diseases account for fully one quarter of the current annual health expenditure. In particular, acute coronary syndrome (ACS) is a leading cause of death in the Western world. A common element among many of the most prevalent cardiovascular conditions is the formation of atherosclerotic plaque, with all its varied biochemical and pathophysiological consequences. Since the effectiveness of treatment falls exponentially from the time of a myocardial event, the ability to rapidly and accurately diagnose cardiovascular pathology, and thereby commence appropriate treatment at a much earlier stage, is critical in reducing the number of deaths from heart disease.

An additional medical benefit to be derived from improved diagnostic screening is the ability to detect patients at risk of developing atherosclerotic lesions and subsequent cardiovascular (and cerebrovascular) pathology. Finally, the development of early and accurate diagnostic tests will enable

health services to reduce the number of unnecessary hospital stays, providing significant cost savings.

The thromboxanes are compounds derived from prostaglandin endoperoxides that cause platelet aggregation, arterial contraction and many other biological effects. One such compound, thromboxane A_2 (TXA_2), a highly unstable biologically active bicyclic oxitane-oxane compound, displays very potent vasoconstricting and platelet aggregating properties. Thromboxane A_2 has been found to play a crucial role in atherothrombotic disorders, and increased synthesis thereof has been found to occur immediately following events such as unstable angina and acute myocardial infarction [Fitzgerald, D. J., et al. (1986), N. Engl. J. Med. 315:983-989]. As mentioned above, thromboxane A_2 is very unstable, and is rapidly converted to stable metabolites, such as TxB_2 and 11-dehydrothromboxane B_2 and 2,3-di-northromboxane B_2 (collectively referred to hereinbelow as "thromboxane B_2" (TXB_2)), which are excreted in the urine. Thus, the presence of thromboxane in urine, released from activated platelets, indicates ongoing platelet aggregation and clot formation.

It has been found that the information derived from the determination of the concentration of thromboxanes in urine samples may be used as a powerful diagnostic tool in patients suspected of suffering from cardiac disease, in particular, acute cardiac syndrome. Consequently, the present invention is primarily directed to an immunoassay method and a new device for quantitative determination of thromboxane in urine in order to diagnose cardiac disease in a subject.

USP 5,047,354 discloses and claims the use of thromboxane to provide an early indication of coronary artery thrombosis by determining the level of TXB_2 or a metabolite thereof or of TXA_2 and comparing said levels with normal levels. Said levels are measured by various methods, e.g., an immunoassay.

Immunoassays are used in the art for the quantitative determination of target substances, having a diagnostic or therapeutic significance, in body fluids. For this purpose, the target substance - antigen - is brought into contact with an antibody that is specific against it or a number of such antibodies and the result of their interaction is measured by any one of the number of methods available in the art. One such method is the amperometric method.

It is known that immobilisation of the components of immune reaction (i.e., antigens and antibodies) on insoluble support gives significant advantages for their detection in complex mixtures like serum. Immobilised proteins retain their activity over long periods of time.

D. Ivnitzki and J. Rishpon in "A one-step, separation-free amperometric enzyme immunosensor", Biosensors & Bioelectronics, Vol. 11, No. 4, pp. 409-417, Elsevier Science Limited, 1996, describe a one-step, separation-free, amperometric enzyme immunosensor which comprises an antibody electrode and an amplification system. The immunological reactions are monitored electrochemically in situ, and the binding curves are directly visualized on a computer screen. This reference will be referred to hereinafter as Ivnitzki et al.

J. Rishpon and D. Ivnitzki in "Amperometric Enzyme-channeling Immunosensor", Enzyme Engineering XIII, Vol. 799 of the Annals of the New York Academy of Science likewise describe enzyme immunoassays (EIA) and apparatus for carrying them out. This reference will be referred to hereinafter as Rishpon et al.

In these immunoassays, an antibody specific against the antigen that constitutes the target substance, and an enzyme, e.g., glucose oxidase (GOX), are immobilised using a glutaraldehyde linker in a polyethylene imine (PEI) layer created on a graphite electrode surface, and brought into contact with a solution consisting of the body fluid to be tested, to which have been added a peroxidase-labeled antibody conjugate and chemicals which form a redox

system together with the aforesaid enzyme. While these are specific examples of immunoassays, different ones can be carried out, in which an enzyme and an antibody are preferably immobilised on a solid phase support.

It has been found that the efficiency of the immunoassays is sharply dependent on the type of solid phase support used for immobilising the enzyme and the antibody.

Immobilisation could be achieved by non-covalent adsorption on plastic or on nitrocellulose membranes or by covalent linkages, like immobilisation on CNBr-Sepharose, diazo-derivatives of cellulose or oxidised cellulose.

Oxidised celluloses (or oxycelluloses) are water insoluble materials produced by reacting cellulose with an oxidant such as gaseous chlorine, hydrogen peroxide, peracetic acid, chlorine dioxide, nitrogen dioxide (dinitrogen tetraoxide), persulfates, permanganate, dichromate-sulfuric acid hypochlorous acid, hyperchlorous acid, hypohalites or periodates. These oxidised celluloses may contain carboxylic aldehyde and/or ketone functional groups, in addition to the hydroxyl groups, depending on the nature of the oxidant and the reaction conditions used in their preparation. The preparation of oxidised celluloses suitable for use as carrier vehicles in the development of cosmetic and pharmaceutical preparations, using alkali metal or alkaline earth metal hypohalites, is discussed in USP 4,480,089. Also, USP 5,780,618 discloses a method for making an oxidised cellulose product from a cellulose material, which involves oxidising a cellulose material into an oxidised cellulose product.

USP 4,405,715 discloses enzymes which are immobilised on a solid support material, containing essentially cellulose and lignin, by a process involving oxidation of the support to provide aldehyde groups, animation of the oxidised support by reacting a diamine with the aldehyde groups, reduction of the aminated support to produce stabilized aminated groups, activation of the aminated groups by reacting the groups with a dialdehyde and immobilisation

of an enzyme by covalent coupling of the enzyme to the activated groups of the support.

Immunoassays have been described in US Patents 4,447,564, 4,654,532, 4,716,121 and 4,582,809, and WO93/20240, which are all incorporated herein by reference. In one example, a "dipstick" format having a polycarbonate detection membrane fused to a polyvinyl chloride sheet is used to provide an immunoassay method usable under field conditions. C.L. Penny et al. [J. Immunol. Methods, 123:185-192 (1989)] describe a method in which the test substance is bound to a detection membrane by immersing such a dipstick into the test sample, which must be a fluid. In a second step, the dipstick is immersed into a solution containing an antibody, to which is conjugated a means of detection, which is then immobilised by immunological reaction with the bound antigenic substance. In a final step, the means of detection is executed, which entails immersing the dipstick into a solution containing the appropriate detection reagents. The assay is described as requiring more than one hour to complete, not including pre-assay sample preparation.

The apparatus described in the art for effecting immunoassays are laboratory apparatus and are not adapted for use by physicians in hospitals or private clinics, particularly for evaluating patients in the emergency department, rapidly to identify those who require urgent therapy.

In co-pending International Application Number PCT/IL00/00650 filed by applicants, a system and corresponding method are provided for carrying out an amperometric immunoassay of a target antigen (thromboxane B_2 (TXB_2) or a metabolite thereof or of thromboxane A_2 (TXA_2).

The system comprises:

- a solid phase support comprising an oxidised cellulose paper comprising immobilised thereon an antibody that specifically binds to said target antigen and at least one enzyme;
- reaction chamber in communication with said paper;

- receiving means for providing the reaction chamber with a test fluid comprising said target antigen and the chemicals required for the particular immunoassay reaction and a redox reaction occurring in response thereto to be carried out;
- at least one electrode juxtaposed with said paper; and
- suitable electronic measuring means operatively connected to said at least one electrode for monitoring a response of said at least one electrode to said redox reaction that occurs in the reaction chamber.

While providing advantages over prior systems, this system has a number of drawbacks. For example, the embodiments described are not adapted for enabling the immunoassay to be easily automated. The embodiments all rely exclusively on using oxidised paper as the solid state support and this limits the applications. In all embodiments, all the chemicals required for the immunoassay are provided together with respect to the paper, and it is not possible to remove any of the contents from the reaction chamber before introducing another chemical therein. Thus, washing solutions cannot be introduced, and the presence of unconjugated antigens provides an artificial increase in the concentration measured. In one embodiment three chemicals may be added separately to the reaction chamber, but the means for so doing are prone to lead to leakages, and as mentioned before, the reaction chamber merely accumulates the chemicals therein.

An important consideration in an amperometric immunoassay is that the concentration results obtained therewith are heavily influenced by dilution level of the sample, such as urine, i.e., if the patient happens to drink a quantity of water before the urine sample is collected this would artificially reduce the concentration of the thromboxane measured. Accordingly, the concentration results obtained in any such immunoassay need to be normalised with respect to the urine dilution. Prior art methods for establishing this normalisation is by determining the osmolarity of the urine or the creatinine concentration therein, which require a separate test for

performing either evaluation, and the subsequent combining of the results with the results of the immunoassay. In applicant's co-pending Israeli Patent Applications Nos. 137307 and 137308, the entire contents of which are incorporated herein by reference, a system is provided for measuring both the thromboxane concentration and the urine electrical conductivity, using an amperometric assay. It was found by applicant that the use of urinary conductivity determination as means for normalising urinary analyte concentrations leads to a significant increase in diagnostic reliability and accuracy.

It is a purpose of the present invention to provide an improved reaction vessel and system, preferably in kit-form, that permits rapid determination by immunoassays of target substances having diagnostic or therapeutic significance.

It is a further purpose of this invention to provide such a reaction vessel and system that is particularly useful for the determination of thromboxane or its metabolites in body fluids.

It is a further purpose of the present invention to replace the oxycellulose paper with beads coated with antibodies, particularly beads made of protein A agarose, in which the IgG antibodies are coupled to the protein A layer. Among other advantages, preparing protein-coated beads is a relatively simple procedure and enables the preparation of a stock solution, resulting in standardisation of measurements.

It is a still further purpose of this invention to provide such a system which is partly disposable, comprising a reaction vessel that can be used typically for only one test and then discarded, while another part, of greater complication and cost, can be retained and used repeatedly.

It is a still further purpose to provide such an apparatus that is simple, of safe operation and inexpensive.

It is a still further purpose to provide such an apparatus that permits determination of other physical parameters of body fluids, in particular conductivity.

Other purposes and advantages of the invention will appear as the description proceeds.

Summary of the Invention

The present invention relates to a reaction vessel for carrying out at least one step of a chemical process and for enabling at least one parameter correlated thereto to be measured, comprising:-

at least one upper holding chamber for containing one or more chemicals needed for said process, each said holding chamber having a first lower outlet means, and further having an upper inlet means for enabling fluid communication with an outside of said reaction vessel;

first plugging means for selectively opening and sealing said upper inlet means;

a reaction chamber for carrying out said process, said reaction chamber being in fluid communication with said at least one holding chamber via said first lower outlet means, said reaction chamber having at least one vent for enabling fluid communication with an outside of said reaction vessel, said reaction chamber further comprising monitoring means for enabling said at least one parameter to be measured;

a lower waste chamber in fluid communication with said reaction chamber via a screen element, said waste chamber having an upper second outlet means for providing fluid communication with an outside of said reaction vessel;

second plugging means for selectively opening and sealing second outlet means;
wherein said first outlet means is adapted to selectively prevent and allow liquid communication therethrough according to whether said upper inlet means is sealed or open, respectively; and wherein said screen member is adapted to selectively prevent and allow liquid communication therethrough according to whether said second outlet means is sealed or open, respectively.

Typically, the first outlet means is adapted to selectively prevent and allow liquid communication therethrough according to whether said upper inlet means is sealed or open by comprising a cross-sectional flow area of magnitude below a predetermined first value, typically about 0.8 square mm.

Similarly, the screen member is adapted to selectively prevent and allow liquid communication therethrough according to whether said second outlet means is sealed or open, by comprising a net-like structure having an open area/total area ratio below a predetermined second value, typically, about 1%.

In particular, the said chemical process comprises an amperometric immunoassay and said at least one parameter comprises an electrical current measurement indicative of a concentration of a predetermined target antigen in a test solution on which said process is carried out. The monitoring means may comprise suitable electrode means in communication with said reaction chamber. The reaction chamber comprises a lateral portal, said electrode means being mounted externally to said reaction chamber and abutting said portal such as to provide communication between said electrode means and the said reaction chamber. Typically, the electrode means comprises a suitable first anode and a suitable cathode in communication with said reaction chamber, said first anode and a cathode each having electrical connectors that are electrically connectable with respect to an external monitoring apparatus, said first anode and said cathode being adapted for measuring a current of said test solution when in contact therewith.

Preferably, the electrode means is further adapted to provide a measurement of conductivity of a suitable liquid accommodated in said reaction chamber and in communication therewith. Thus, the electrode means may further comprise a second anode, said second anode having an electrical connector that is electrically connectable with respect to an external monitoring apparatus, said second anode and said cathode being adapted for measuring a conductivity of said test solution when in contact therewith.

The reaction vessel may comprise a plurality of said upper holding chambers, typically between 3 and 7, and preferably 5, each said holding chamber having a corresponding said first lower outlet means in communication with said reaction chamber. The plurality of holding chambers are typically arranged circumferentially about a longitudinal axis of said reaction vessel.

The reaction vessel further comprises an upper lumen coaxial with said longitudinal axis and extending from an uppermost part of said reaction vessel to said reaction chamber.

The corresponding upper inlet means of said plurality of said holding chambers are typically substantially coplanar and substantially equally displaced from said longitudinal axis of said reaction vessel in a radial direction. Also, the second outlet means is typically substantially coplanar with said upper inlet means, and is substantially equally displaced from said longitudinal axis of said reaction vessel in a radial direction as said upper inlet means. Each corresponding first plugging means may comprise a resilient tongue element radially disposed with respect to said longitudinal axis and cantilevered at an outer radial end thereof with respect to a common supporting ring, said tongue element comprising a tab at an inner radial free end thereof, and a stopper at a lower face of said tongue adapted for closing or opening the corresponding said upper inlet means when the corresponding free end of said tongue element is pressed thereagainst or distanced therefrom, respectively. Similarly, the second plugging means may comprise a resilient tongue element radially disposed with respect to said longitudinal axis and cantilevered at an outer radial end thereof with respect to said common

supporting ring, said tongue element comprising a tab at an inner radial free end thereof, and a stopper at a lower face of said tongue adapted for closing or opening the said second outlet means when the corresponding free end of said tongue element is pressed thereagainst or distanced therefrom, respectively. The tabs of said first plugging elements and the tab of said second plugging element are each displaced radially from said longitudinal axis typically by a substantially equal first radial displacement.

The reaction vessel typically further comprises suitable sequencing means for selectively opening or closing each said first plugging element and said second plugging element in response to a predetermined angular rotation of said sequencing means. The sequencing means may comprise an assembly of a sequencing portion joined for rotation to a first actuating portion and rotatably mounted with respect to said reaction vessel coaxially with said longitudinal axis, said sequencing portion being adapted for selectively opening or closing any one of said first plugging elements and said second plugging element in response to a predetermined angular rotation of said first actuating portion. The sequencing portion may comprise a cam element disposed at a circumferential location with respect to said longitudinal axis, such as to raise a said tab when said cam is angularly aligned with respect to the tab such as to open a corresponding said upper inlet means or said second outlet means. The resilient tongues may be biased to close the corresponding said upper inlet means or said second outlet means when the corresponding tab is not circumferentially aligned with said cam element. Optionally, the sequencing portion further comprises an upper annular lip adapted for pushing downwardly any said tab that is not circumferentially aligned with said cam element such as to close the corresponding said upper inlet means or said second outlet means. Further, the sequencing assembly comprises a central aperture extending therethrough longitudinally.

The reaction vessel preferably further comprises a common supporting ring, wherein each said first plugging means and said second plugging means are

joined at their outer radial ends to said supporting ring. Optionally, the supporting ring is integrally joined to said first plugging means and to said second plugging means. The said supporting ring, each said first plugging means and said second plugging means may be made from silicone. Alternatively, the supporting ring, and said tongue element of each said first plugging means and said second plugging means is made from a suitable metal including stainless steel, and wherein each said stopper of each said first plugging means and said second plugging means is made from silicone or any other suitable material. Optionally, the plugging means may be in the form of an O-ring.

The reaction vessel typically further comprises a stirrer assembly having a shaft rotatably mounted with respect to said central lumen and said central aperture, said shaft having a stirring portion at one end thereof extending into said reaction chamber, and a second actuating portion at the other end thereof extending outside of said reaction vessel.

Each said first plugging means and the second plugging means may be made from any suitable material including silicone.

Optionally, the reaction chamber and the waste chamber are comprised in a lower portion, and the holding chambers, sequencing means, stirrer assembly, first plugging means and second plugging means are comprised in an upper portion, wherein said upper portion is selectively mountable and optionally dismountable with respect to said lower portion.

Preferably, the reaction vessel is substantially disposable.

At least a suitable antibody appropriate for said immunoassay is immobilised in a solid state support provided in the reaction vessel. The antibody may be provided from any suitable source, and may include at least one of human antibodies, animal antibodies, and artificial antibodies such as for example as may be obtained from molecular imprinting processes.

The solid state support preferably comprises a plurality of beads coated with a suitable protein. The beads may be provided in the reaction chamber prior to

commencing said immunoassay. Alternatively, the beads may be provided in one of the plurality of holding chambers, and wherein the beads may be delivered therefrom to the reaction chamber operative to a predetermined actuation of the sequencing assembly. Alternatively, the beads may be provided in the central lumen, wherein the beads may be delivered therefrom to said reaction chamber operative to the test solution being delivered to the reaction chamber via the central lumen. The central lumen also acts as the vent for the reaction chamber.

Alternatively, the solid state support may comprise a layer of a suitable protein provided in an inner wall of said reaction chamber.

Alternatively, the solid state support may comprise a suitable oxidised cellulose paper in said reaction chamber.

The present is also directed at a system for carrying out an amperometric immunoassay on a test solution having a target antigen, comprising:-

a reaction vessel as described, selectively mountable and dismountable with respect to;

suitable electronic control and measuring means for controlling operation of said reaction vessel and for monitoring the response of the electrode means.

Preferably, the control and measuring means are comprised in a suitable housing, which may further comprise an operations chamber adapted for accommodating therein the reaction vessel during operation thereof.

The system comprises suitable first and second actuation means engageable for rotation with said first actuation portion and second actuation portion, respectively, of the reaction vessel, when the reaction vessel is mounted to the control and measuring means. The control and measuring means is adapted for enabling operative electrical contact with electrode means of the reaction vessel such as to enable electrical current and conductivity measurements of a test solution accommodated in said reaction chamber to be performed. Thus, the electrode means preferably comprises an outwardly extending tip with respect to said reaction vessel. Similarly, the control and measuring means

preferably comprises a terminal operatively connected thereto and adapted for engaging with said tip such as to establish electrical communication therewith when said reaction vessel is mounted to said control and measuring means. Typically, the control and measuring means comprises suitable amperometric circuit means for measuring an electric potential of the said electrode means, and also further comprises means for digitally processing a change in said electric potential generated in response to said reaction to provide a first signal representative of the immunoassay.

The control and measuring means typically further comprises means for digitally processing a change in said electric potential generated in response to said reaction to provide a second signal representative of the conductivity of the test solution, and may also further comprise suitable memory means for storing comparison values used for enabling calculation of a desired parameter representing the result of the assay from at least one of said first signal and said second signal, and processing means for carrying out such calculation. The said parameter is typically target concentration data, and preferably normalised target concentration data.

The system preferably further comprises a suitable electronic display adapted for displaying said parameter, and the display may be comprised in the control and measuring means.

In the system according to the present invention, each upper holding chambers may comprise suitable chemicals required for the particular immunoassay being performed. Typically, the holding chambers comprise an oxidase-labeled antibody-antigen conjugate, wherein the antigen is the same as the analyte of interest, and components required to create a redox system with the aforesaid oxidase.

The oxidase-labeled antibody-antigen conjugate may be horseradish peroxidase-labeled antibody-antigen conjugate.

The system may further comprise suitable portable power means, including, for example, at least one electrical battery.

Typically, the antibody immobilised in the solid substrate is that required for the determination of TXB_2 or any one of the metabolites thereof, or any one of the metabolites of TXA_2.

Brief Description of the Figures

Figures 1(a), 1(b) and 1(c) illustrate, in transverse cross-sectional view, the main elements of a first embodiment of the present invention, illustrating operation thereof.

Figure 2 illustrates, in partially exploded perspective view, a second, and preferred, embodiment of the present invention.

Figure 3 illustrates, in plan view, the embodiment of Figure 2.

Figure 4 illustrates, in transverse cross-sectional view, the embodiment of Figure 3 taken along C-C.

Figure 5 illustrates, in transverse cross-sectional view, the embodiment of Figure 3 taken along B-B.

Figure 6 illustrates, in plan cross-sectional view, the embodiment of Figure 5 taken along D-D.

Figure 7 illustrates the main elements of the electrode means of the embodiment of Figures 2 to 6.

Figure 8 shows in transverse cross-section a representative plugging means of the embodiment of Figures 2 to 6.

Figure 9 shows in perspective view the sequencing portion of the embodiment of Figures 2 to 6.

Figure 10 shows in perspective view the sequencing portion and plugging assembly of the embodiment of Figures 2 to 6.

Figure 11 shows in perspective view a preferred embodiment of the system of the present invention, with the reaction vessel removed from the operations chamber of the electronic control and measuring means.

Figure 12 shows in perspective view a preferred embodiment of the system of the present invention, with the reaction vessel removed mounted in the operations chamber of the electronic control and measuring means.

Description

The present invention is defined by the claims, the contents of which are to be read as included within the disclosure of the specification, and will now be described by way of example with reference to the accompanying Figures.

One aspect of the present invention is a system for carrying out amperometric assays, particularly immunoassays, of a target antigen, in which chemicals required in said assay are provided in an automated manner, such chemicals including particularly an enzyme, for example but not exclusively an oxidase, e.g. glucose oxidase (GOX) and HRP, and an antibody specific against the target antigen, and immobilised in solid phase support. For the purpose of illustration, the remaining features of the assay, are similar to those described by Rishpon et al., but it will be understood that the invention is not limited to such features, but extends to any application in which an enzyme and an antibody are immobilised in a solid phase support for amperometric assays.

The present invention further extends to the application of such a system for performing any amperometric assay that comprises monitoring the potential of an electrode resulting from a reaction that involves reagents immobilised in a solid phase support.

The efficiency of the solid phase supports is defined, for the purposes of this invention, by the antigen-binding activity of anti-bodies immobilised therein. For the particular purpose of amperometric immunoassays, the efficiencies of different supports can be compared by comparing the potentials of a given electrode in a given apparatus and in tests of a given solution.

The present invention relates in particular to a system for performing amperometric immunoassays, comprising a reaction vessel selectively mountable to an apparatus. The reaction vessel is preferably a disposable part,

comprising suitable means for carrying out the immunoassay, and a non-disposable part for analysing and displaying the results of the assay, as is described hereinbelow in greater detail. In a wider sense, the reaction vessel, and the system, of the present invention may also be adapted for performing a process or reaction in a simple and straightforward manner, wherein a number of chemicals need to be provided separately to, and removed from, a reaction chamber, typically in a particular sequence, the value of a measured parameter being indicative of the result of such a reaction or process.

Thus, in its widest aspect, and referring to Figures 1(a) to 1(c), the present invention, in a first embodiment thereof, relates to a reaction vessel (10) for carrying out a chemical process or reaction comprising a housing (15) having at least one, and preferably more than one separate, upper holding chambers (20) for containing flowable contents (30), typically one or more chemicals needed for the process or reaction. Each holding chamber (20) has an upper opening (22), which is selectively and reversibly sealable with respect to an outside (500) thereof via a suitable plugging element (26). Each holding chamber (20) has sufficient volumetric capacity to hold therein the required volume of contents, typically in liquid form, preferably including a headspace (28), and has a lower fluted end with one or more relatively narrow conduits or lower apertures or openings (50) to provide fluid communication with a lower processing or reaction chamber (40). The reaction chamber (40) is in communication with a lower, waste chamber (60) via a retaining screen member (45) at the lower end of the reaction chamber (40). The reaction chamber (40) further comprises a vent (85) for maintaining open fluid communication between the reaction chamber (40) and an outside, typically said outside (500). The housing (15) further comprises a flue (70) having a lower end (74) thereof in communication with the waste chamber (60). An upper end (72) of the flue (70) is selectively and reversibly sealable with respect to an outside, typically said outside (500), via a suitable plugging element (76).

The plugging elements (26) and (76) may comprise any suitable means for enabling the respective openings (22) and (70) to be selectively sealed or opened. Typically, said plugging elements (26) and (76) may comprise an integral silicone cantilevered sealing member, as illustrated in Figure 1(a) to 1(c), or may each comprises a plastic or metal (preferably stainless steel) cantilevered member having a plugging element such as for example an O-ring or silicone or rubber disc at an extremity thereof adapted to match and seal against the respective opening.

Each lower opening (50) is of a suitable size or gauge such that natural flow of the contents of the holding chamber (20) into the reaction chamber (40) is prevented when the upper opening (22) is sealed, while allowing these contents to flow into the reaction chamber (40) when the upper opening (22) is opened via said plugging element (26). Thus, when the upper opening (22) is closed, the contents (30) of the holding chamber (20) are prevented from naturally flowing into the reaction chamber (40) since this would cause a reduction in the air pressure within the headspace (28) of the chamber (20). This phenomenon has the effect of keeping the contents within the holding chamber (20), even though the lower end thereof is open via said lower opening (50). Any tendency for the contents (30) to flow out is counterbalanced by a corresponding increase in the suction provided in the headspace (28) due to a lowering of the air pressure therein that would result from the expansion of the volume thereof due to the outflow of the contents (30) from the holding chamber (20). Disadvantageously, if the lower opening (50) were too large in cross-sectional flow area, there is a possibility of an air bubble traversing it upwardly from the reaction chamber (40), which would result in displacement of some of the contents (30) downwards into the reaction chamber (40), even though the upper opening (22) is still closed. In normal operation of the vessel (10), and as illustrated in Figure 1(b), when the plugging element (26) is removed from and effectively unseals the upper end (22) of the holding chamber (20), air is allowed into the holding chamber (20) from outside (500) and thus enables the contents (30) to flow downwards under gravity into the

reaction chamber (40). The air in the reaction chamber (40) displaced by the liquid contents (30) flows to the outside (500) via vent (85). Typically, the said lower opening (50) is about 1mm in diameter, but may be greater or less than this value.

Similarly, the screen member (45) is of a suitable configuration such that that natural flow of the contents of the reaction chamber (40) into the waste chamber (60) is prevented when the upper end (72) of the flue (70) is sealed, while allowing these contents to flow into the waste chamber (60) when the upper end (72) is opened via said plugging element (76). Typically, the screen member (45) comprises a plurality of small apertures through which the liquid contents (30) of the reaction chamber (40) may pass into the waste chamber (60) when there is no resistance to do so from the waste chamber (60), arising from the air trapped therein. In other words, when the upper end (72) is open, the air in the waste chamber (60) is able to escape from the waste chamber (60) as it is displaced by the liquid contents (30) flowing into it from the reaction chamber (40). However, when the upper end (72) is closed and air from the waste chamber (60) is unable to flow out therefrom, there is a certain resistance to flow of the liquid contents from the combustion chamber (40) into the waste chamber (60). The reason for this is that air from the waste chamber (60) cannot be displaced upwards into the reaction chamber (40) because of the small size of the apertures comprised in the screen member (45), which are in any case effectively "blocked" by the presence of the liquid contents (30) therein; if liquid were to flow into the waste chamber (60) the air in this chamber would be compressed accordingly to accommodate the liquid volume, thus leading to resistance. The pressure head provided by the liquid in the reaction chamber (40) is not enough to enable the liquid to force itself into the waste chamber against such resistance. However, once the upper opening (72) is opened, the pressure head of the liquid is sufficient to enable the liquid to flow downwards, since the displaced air in the waste chamber (60) can be vented out via the flue (70) and upper opening (72). Typically, the surface tension of the liquid within the apertures of the screen member (45) has the

effect of providing a barrier through which the rest of the liquid in the reaction chamber (40) cannot penetrate until the pressure differential across the screen member (45) reaches a predetermined minimum, achieved when the upper opening (72) is opened to the atmosphere.

Thus, the screen member (45) may comprise a permeable membrane, or a porous film or a mesh or net-like structure, or any other suitable structure adapted for functioning as described herein, and thus enable the liquid contents of the reaction chamber (40) are able to flow through small apertures or the like comprised therein under a predetermined pressure head differential across the screen member (45). Said screen member (45) may comprise, for example, a screening fabric made by Sefarnitex(ref. 03-6/5) having a mesh open area of about 0.75% (of the total screen area), warp/weft ratio of (165 per cm)/(270 per cm).

In normal operation of the vessel (10), and as illustrated in Figure 1(c), when the plugging element (76) is removed from and effectively unseals the end (72) of the flue (70), air is allowed to exit the waste chamber (60) to the outside (500) and thus enables the contents of the reaction chamber (40) to flow downwards under gravity into the waste chamber (60), as the air therein is displaced to the outside. At the same time, air from the outside enters the reaction chamber (40) via vent (85) to occupy the volume vacated by the liquid contents (30).

The holding chamber (20) may comprise all the necessary chemicals required for a particular process or reaction within the reaction chamber (40), that may be activated by any method or means therein. For example, the reaction chamber (40) may comprise a substance with which the contents of the holding chamber (20) are to react. Alternatively, the reaction chamber (40) may provide a suitable window for irradiating the reaction chamber with any desired radiation to provide a reaction of the contents of the holding chamber (20) when these are placed in the reaction chamber (40), the other parts of the

vessel (10), in particular the holding chamber (20), being substantially opaque to such irradiation. Alternatively, and preferably, the vessel (10) comprises a plurality of holding chambers (20), each of which comprises a particular chemical, including reagents, test solutions, buffer solutions, rinsing solutions and so on, each of which may be selectively delivered to the reaction chamber (40) in a desired sequence via their corresponding lower openings (50) as desired, and individually evacuated from the reaction chamber (40) into the waste chamber (60) as desired, to execute a particular process or set of reactions.

The reaction chamber (40) is adapted for carrying out a process or a reaction, and for enabling one or more parameters indicative of the result of the process or the reaction to be detected or monitored. In some embodiments, the result of a desired reaction may be a colour change of a compound in the reaction chamber (40), and this may be detected and measured using any suitable optical device via a suitable window (not shown). Alternatively, and preferably, the result of a desired reaction provides a change in the electrical potential within the reaction chamber (40), and this may be monitored and measured via a suitable electrode arrangement (80) that is in electrical communication with the reaction chamber (40). The electrode arrangement (80) is connected to any suitable electronic equipment, including for example a suitable microprocessor computer, enabling analysis of electrical signals received from the electrode arrangement (80).

In the second, and preferred, embodiment according to the present invention, the reaction vessel (110) is particularly adapted for carrying an immunoassay therein, specially an amperometric immunoassay.

Thus, in the preferred embodiment, and referring to Figures 2 to 10, in particular Figure 2, the reaction vessel (110) comprises an upper portion (200) and a lower portion (300). Advantageously, as will be explained in greater detail below, the lower portion (300) is selectively mountable and dismountable

with respect to the upper portion (200), but in other embodiments these two portions may be suitably joined or bonded one to the other, reversibly or permanently, or indeed constructed as a unitary integrated device.

Referring particularly to Figures 2 to 6, the lower portion (300) comprises a housing (325) that is typically, but not necessarily, cylindrical in form, having a central lumen (310) substantially coaxial with the central longitudinal axis (390) of the lower portion (300). The lumen (310) has an upper open end (315) and provides communication between this and a reaction chamber (340) disposed below the lumen (310). The reaction chamber (340) is in fluid communication with a lower waste chamber (360) via a suitable screen member (345), and these features are similar in form and function to the reaction chamber (40), waste chamber (60) and screen member (45), respectively, as described hereinbefore with respect to the first embodiment, mutatis mutandis.

Optionally, the screen member (345) may be held in place via push ring (346) and O-ring seal (347) which form a tight fit against a downwardly extending annular recess (342) formed between the reaction chamber (340) and the waste chamber (360). In other embodiments, the screen member (345) may be integrally formed in the reaction chamber (340), for example, or may be attached thereto in any convenient manner, including heat welding or bonding, ultrasonic welding, or via suitable adhesives, for example, with or without a push ring (346).

A plurality of gutters, in the illustrated embodiment five gutters, are formed in the upper part of the lower portion. These gutters, designated (350A), (350B), (350C), (350D) and (350E), are circumferentially disposed with respect to the central longitudinal axis (390), and each said gutter extends in a downward and radial direction from near the upper periphery of the lower portion (300) towards the lumen (310), and are thus in fluid communication therewith.

A flue (270) in the form of a tubing provides communication between the waste chamber (360) and the outside environment (500), the flue (270) having a lower end (274) that opens into the waste chamber (360), and an open upper end (272). The flue (270) is typically arranged circumferentially between gutters (350A) and (350E). As will become clearer below, an upper portion (273) of the flue (270) comprising the upper end (272) extends upwardly from the lower portion (300) in the form of a tube, the upper end (272) being selectively and reversibly sealable with respect to an outside, typically said outside (500) via a suitable plugging element.

As illustrated in Figures 4 and 5, the lower end of the waste chamber (360) comprises a bottom end cap (365) joined thereto. Preferably, though, the end cap (365) is formed integrally with the lower portion (300).

The reaction chamber (340) comprises a typically circular window (349) providing communication between the reaction chamber (340) and a typically rectangular slot (375) formed in the external part of the housing (325), typically along a chord perpendicular to the axis (390). Referring also to Figures 2 and 4, the slot (375) is adapted to receive an electrode means (600). The electrode means (600) is typically, but not necessarily, in the form of a printed item, comprising a substrate (610), wherein a first anode (630), a cathode (640) and a second anode (650) are printed thereon by suitable means. The cathode (640), also known as the working electrode, is typically in the form of a disc having a suitable electrical contact (645) extending therefrom. The first anode (630), also known as a counter electrode, comprises a relatively large surface area and is advantageously in the form of a crescent arranged around the cathode (640), for compactness, and has a suitable electrical contact (635) extending therefrom. The second anode (650), also known as the reference electrode, is preferably circumferentially aligned with the first anode (630), and has a suitable electrical contact (655) extending therefrom. The first anode (630) and the cathode (640) are used to measure the electrical conductivity of the liquid contained in the reaction chamber (340), typically a

urine sample, while the second anode (650) and the cathode (640) are used for the amperometric measurements of the immunoassay. The electrode means (600) is sealingly accommodated in said cavity (375) such as to prevent leakage into or out of the reaction chamber (340), and such that the electrodes (630), (640) and (650) are electrically exposed to the inside of said reaction chamber (340) via said window (349). An end portion (660) of the electrode means (600) comprising the contacts (635), (645) and (655) extends laterally beyond the said housing (325).

Referring in particular to Figures 2, 4 and 6, the upper portion (200) comprises a housing portion (215) having plurality of holding chambers formed therein, typically five holding chambers as illustrated, designated herein as (20A), (20B), (20C), (20D) and (20E), each of which having a lower opening, (50A), (50B), (50C), (50D) and (50E), respectively, and each being correspondingly similar in structure and function to the holding chamber (20), and lower opening (50), respectively, as described for the first embodiment, mutatis mutandis. The holding chambers (20A), (20B), (20C), (20D) and (20E) are arranged circumferentially about the central axis (290) of the housing portion (215), which typically has a substantially cylindrical external profile, such as to provide registry between each corresponding lower opening, (50A), (50B), (50C), (50D) and (50E), respectively, thereof and a corresponding said gutter (350A), (350B), (350C), (350D) and (350E), respectively, when the upper portion (200) is coaxially aligned with and mounted onto said lower portion (300).

The volumetric capacities of the reaction chamber (340) and of the waste chamber (360) are each typically at least equal to the combined volume of all the said holding chambers (20A), (20B), (20C), (20D) and (20E).

The housing portion (215) further comprises a central lumen (210) extending the longitudinal length thereof. A spinner or stirrer assembly (700) is comprised in said upper portion (200), having a shaft (710) rotatably mounted

within said lumen (210). The stirrer assembly (700) comprises a spinning or stirring portion (720) at the lower end of the shaft (710) and extending beyond the lower part of the lumen (210), and an actuating portion (730) at the upper end thereof of the shaft and extending beyond the upper part of the lumen (210). The stirring portion (720) is adapted to be accommodated within at least a portion of the lumen (310) and reaction chamber (340) of the lower portion (300) when this is mounted with respect to the upper portion (200). Further, the stirring portion (720) comprises vanes, paddles, rivulets or any other suitable feature that provides a stirring motion to the contents within the reaction chamber (340) when rotated about axis (290). The actuating portion (730) comprises in this embodiment a gear wheel coaxially aligned with and fixed to the shaft (710), such as to enable rotation therewith. As will become clearer hereinbelow, a suitable external rotation means such as an electrical motor provides rotation to the stirring assembly (700) via, for example, a worm gear, another gear wheel or a drive belt suitably rotatingly engaged with the actuating portion (730) to provide rotational motion to the stirring portion (720). The stirrer assembly (700) further comprises a lumen (715) extending the longitudinal length thereof, and having an optionally closable upper open end (716) via which a sample or any other chemical may be introduced directly to the reaction chamber (340) from the outside (500). The lower end (717) of the lumen (715) may be optionally temporarily closed until first use of the vessel (110). In such a case, the lower end (717) may comprise a film seal which may be adapted to break open under force of introduction of the sample therethrough, for example.

The reaction chamber (340) comprises a vent (385) for maintaining open gaseous communication between the reaction chamber (340) and an outside, typically said outside (500), similar in form and function to the vent (85) as described for the first embodiment, mutatis mutandis. In the second embodiment, the vent (385) is provided by said lumen (715) of the stirrer assembly (700), which thus provides communication between the reaction chamber (340) and the outside (500).

The housing portion (215) further comprises a second lumen (277) radially displaced from the central lumen (210) and circumferentially arranged between holding chambers (20A) and (20E), and adapted to accommodate the upper portion (273) of the flue (270) when the said lower portion (300) is mounted with respect to the said upper portion (200).

The upper portion (200) further comprises an upper plate (295) joined to the body portion (215) and comprising a plurality of upper openings (22A), (22B), (22C), (22D) and (22E), respectively, corresponding to and in registry with said holding chambers (20A), (20B), (20C), (20D) and (20E), respectively. Each upper openings (22A), (22B), (22C), (22D) and (22E), is preferably equidistantly displaced radially with respect to said axis (290), and are similar in structure and function to the upper opening (22), as described for the first embodiment, mutatis mutandis. The upper plate (295) also comprises a central aperture (291) adapted for accommodating therein part of said shaft (710), and another aperture (292) in registry with lumen (277). The flue (270) is radially disposed in said lower portion (300) such that the upper end (272) is equidistant from the axis (290) as the upper openings (22A), (22B), (22C), (22D) and (22E), when the lower portion (300) is mounted with respect to the upper portion (200). Similarly, the longitudinal length of the upper portion (273) of the flue (270) is such that the upper end (272) is substantially co-planar with the upper openings (22A), (22B), (22C), (22D) and (22E), when the lower portion (300) is mounted with respect to the upper portion (200). The upper plate (295) may be integrally joined to the body portion (215), or alternatively removably or permanently joined by any suitable means including, for example, mechanical fixing means, adhesive means, via ultrasonic or heat welding means, and so on.

Each of the upper openings (22A), (22B), (22C), (22D) and (22E), is selectively and reversibly sealable with respect to the outside (500) thereof via a suitable plugging element, (26A), (26B), (26C), (26D) and (26E), respectively, each

similar in function to said plugging element (26) as described for the first embodiment, mutatis mutandis. Similarly, the upper end (272), is also selectively and reversibly sealable with respect to said outside (500) via a suitable plugging element, (276) similar in function to said plugging element (76) as described for the first embodiment, mutatis mutandis. Referring in particular to Figure 8, each plugging element (26A), (26B), (26C), (26D) and (26E), and (276) is similar to one another, and each comprises a resilient tongue element (670) cantilevered at one end thereof with respect to a supporting ring (675) and comprising at the free end (678) of the tongue element (670) a tab (672). A stopper (677) is provided at the lower face of the tongue element (670) at or near the free end (678), adapted for selectively sealingly closing or opening the corresponding upper opening (22A), (22B), (22C), (22D) or (22E), or upper end (272), when the free end (678) is pressed thereagainst or distanced therefrom, respectively.

As illustrated best in Figures 3 and 10, in the preferred embodiment, the plugging elements (26A), (26B), (26C), (26D), (26E) and (276) are co-planarly joined, preferably integrally, to a common supporting ring (675) to form a plugging assembly (679). Each corresponding tongue element (670) extends substantially radially inwards towards the centre of the ring (675). Preferably, the support ring (675) comprises an upper annular disc portion (674), preferably integrally joined thereto, to provide improved mechanical properties. The plugging assembly (679) is adapted to fit over the top plate (295) and upper parts of the housing portion (215), wherein the corresponding stopper (677) of each said plugging elements (26A), (26B), (26C), (26D), (26E) and (276) is in vertical registry with their corresponding upper opening (22A), (22B), (22C), (22D) or (22E), or upper end (272), respectively. The plugging assembly (679) is preferably a unitary item, made from a suitable resilient material including silicone, for example. Alternatively, the plugging assembly may be made from a suitable plastic or metal, such as for example stainless steel, wherein the tab (672) of each tongue element (670) may be made from a sealing material such as silicone, or alternatively, the tab may also be plastic

or metal, but comprise a suitable O-ring for sealing. Preferably, each tongue element (670) is preferably biased such that in the unstressed state (i.e., when not subject to external forces) the corresponding plugging elements (26A), (26B), (26C), (26D), (26E) and (276) is in sealing mode with respect to the corresponding upper opening (22A), (22B), (22C), (22D) or (22E), or upper end (272), respectively.

In the preferred embodiment, sequencing means are provided enabling the plugging elements (26A), (26B), (26C), (26D) and (26E), and (276) to be selectively and sequentially opened and closed, in a simple and controllable manner. This has the advantage that, correspondingly, the contents of each holding chamber (20A), (20B), (20C), (20D) and (20E), may be individually delivered to the reaction chamber (340), and evacuated therefrom, independently from one another, as will be described in greater detail hereinbelow. Referring to Figure 4, said sequencing means are in the form of a sequencing assembly (800), comprising a sequencing portion (850) joined for rotation to a suitable actuating portion (860), the sequencing portion (850) being adapted for selectively opening or closing each said plugging element (26A), (26B), (26C), (26D) and (26E), and (276), as desired, typically by means of a rotational motion of the sequencing portion (850). The sequencing portion (850) and actuating portion (860) are mounted for rotation with respect to said shaft (710), and are thus concentrically aligned therewith. The actuating portion (860) comprises in this embodiment a gear wheel coaxially aligned with said sequencing portion (850), such as to enable rotation therewith. As will become clearer hereinbelow, a suitable external rotation means such as an electrical stepping motor, for example, provides rotation to the sequencing assembly (800) via, for example a worm gear, another gear wheel or a drive belt suitably rotatingly engaged with the actuating portion (860) to provide rotational motion over any desired angular displacement to the sequencing portion (850). In the preferred embodiment, and referring in particular to Figure 9, the sequencing portion (850) comprises a disc element (852) having a cam element (855) provided at an angular location on the circumferential

periphery (859) thereof. Preferably, a disc-like lower rail (858) is provided on said periphery (859), smoothly dovetailing with respect to the cam element (855) at either side thereof. The sequencing portion (850) further comprises an upper disc-like shoulder (854) having a concave niche (853) complementary shaped with respect to and vertically aligned with said cam element (855). Referring to Figures 4 and 10 in particular, when the sequencing portion (850) is mounted to said upper portion (200), the tabs (672) of the said plugging element (26A), (26B), (26C), (26D) and (26E), and (276), are situated between the lower rail (858) and the shoulder (854). As the sequencing portion (850) is rotated about axis (390) via actuating element (860), the cam element (855) may be selectively and sequentially brought into registry with one tab (672) of only one, in turn, of the plugging elements (26A), (26B), (26C), (26D) and (26E), and (276), thereby pushing the specific tab element (672) in an upwardly direction and thus opening the corresponding upper opening (22A), (22B), (22C), (22D) or (22E), or upper end (272), respectively. At the same time, the shoulder (854) abuts against the upper part of the tab elements (672) so that the remaining upper openings remain closed. Any one of these angular positions of the cam member (855) in which one of the plugging elements (26A), (26B), (26C), (26D) and (26E), and (276) is in open mode is referred to herein as an opening position with respect thereto. The sequencing portion (850) may also be rotated to a particular angular position such that the cam element (855) is circumferentially disposed between two adjacent tab elements (672), in which case all the plugging elements (26A), (26B), (26C), (26D) and (26E), and (276) remain in closed mode. In this latter case, any one of these angular positions of the cam member (855) in which all the plugging elements (26A), (26B), (26C), (26D) and (26E), and (276) are in closed mode is referred to herein as a neutral position. Thus, by rotating the said sequencing assembly (800) by a specific angular displacement, each one of the said upper openings (22A), (22B), (22C), (22D) or (22E), or the said upper end (272) may be opened in turn as desired, or indeed all may remain closed.

During general operation of the reaction vessel (110), a plurality of suitable chemicals, including reagents and washing buffers, are separately contained in holding chambers (20A), (20B), (20C), (20D) and (20E), preferably in the particular order in which they need to be provided to the reaction chamber (340), though alternatively they may be contained in any desired permutation with respect to the holding chambers. Where more than five separate chemicals are required to be delivered separately to the reaction chamber, the reaction vessel is provided with a corresponding number of holding chambers, gutters, plugging elements and so on, as described herein with respect to the preferred embodiment, mutatis mutandis. The sequencing assembly (800) in conjunction with the plugging assembly (679) enable the contents of each of the holding chambers (20A), (20B), (20C), (20D) and (20E), to be delivered to the reaction chamber (340), in turn, via the corresponding lower openings, (50A), (50B), (50C), (50D) and (50E), respectively, thereof and corresponding said gutters (350A), (350B), (350C), (350D) and (350E), respectively. The sequencing assembly (800) in conjunction with the plugging assembly (679) also enable the contents of the reaction chamber (340) to be drained to the waste chamber (360) at any stage, as required.

As mentioned hereinbefore, the lower portion (300) is preferably selectively mountable to the upper portion (200). This has significant storage advantages, particularly when at least one of the holding chambers (20A), (20B), (20C), (20D) and (20E) comprises a chemical that needs refrigeration, for example, to prevent its degradation or denaturing, and thus less space is taken up in a refrigeration unit by the upper portion (200) than would be the case with the whole reaction vessel (110).

In the preferred embodiment, the reaction vessel (110) is preferably disposable, but in other embodiments parts or all of the reaction vessel may be semi-reusable or non-disposable for a host of applications, with suitable cleaning thereof between uses. The term disposable is herein taken to mean "designed to be thrown away after use with only negligible loss". The useful life of the

reaction vessel typically ends with either contamination of the contents therein, or with reaction of the contents within the reaction chamber. Thus the term "disposable" is clearly understood in the context of the present specification to refer to the reaction vessel being designed to be thrown away after use with negligible economic loss, after completion of the immunoassay, for example. In contrast, the non-disposable apparatus, described hereinbelow, of the present invention is designed for many use cycles, each cycle with a new disposable reaction vessel, typically, mounted thereto, rather than being designed to be disposed of the first time that it is used, and may thus constitute a significant capital item in relation to the reaction vessel.

The reaction vessel (110) may be adapted for a host of different applications in which a number of different predetermined chemicals need to be individually and separately delivered to a reaction chamber and removed therefrom, specially in a desired sequence and optionally automatically. In particular, the reaction vessel (110) according to the present invention is adapted for carrying out immunoassays, specially amperometric immunoassays, and thus comprises a suitable means for carrying out amperometric immunoassays with particular sensitivity and efficiency, said means being typically in the form of a solid phase support for immobilising a specific antibody and an enzyme.

Thus, referring to Figures 11 and 12, preferably, and in the preferred embodiment, the reaction vessel (110) is selectively mountable and dismountable with respect to a suitable electronic control and measuring means (900) for controlling the operation of the reaction vessel (110) and for monitoring the response of the electrode (600). The electronic control/measuring means (900) is in operative electrical contact with the electrode (600) when the reaction vessel (110) is mounted on the control/measuring means (900) and the ensuing system (999), comprising said reaction vessel (110) and the control/measuring means (900), is in operation.

The control/measuring means (900) typically comprises a housing (910) having an operations chamber (920) therein adapted for accommodating said reaction vessel (110). A reversibly closable and optionally lockable door (930) is suitably hinged to said housing to provide access to said operations chamber (920) for the reaction vessel (110). Preferably, the door (930) comprises windows (932), (934) to enable safe viewing of the reaction vessel (110) during operations of the system (999). A pair of suitable supports (921), (922) are provided in the operations chamber (920) complementary to parallel slots (121), (122) provided in the said upper portion (200) of the reaction vessel (110). The supports (921), (922), and correspondingly the slots (121), (122) are typically at different heights with respect to the longitudinal axis of the reaction vessel (110), thereby ensuring that the reaction vessel (110) is supported within the operations chamber (920) in an upright position and at the correct orientation. In this way, the position of the each part of the reaction vessel (110), and in particular of the holding chambers (20A), (20B), (20C), (20D) and (20E), relative to the control/measuring means (900) is known and fixed.

In addition, the control/measuring means (900) further comprises suitable actuation means (942), (944) for actuating and controlling actuation of both, the actuating portion (730) of the stirrer assembly (700), and of the actuating portion (860) of the sequencing assembly (800). Thus actuation means (942) typically comprises a suitable electric motor operatively connected to a suitable gear wheel (945) that is meshable with the said actuating portion (730), so that when engaged, the actuation means (942) provides rotational motion to the stirrer assembly (700) when required, at controlled RPM and rotational direction. Similarly, actuation means (944) typically comprises a suitable electric motor, preferably a stepping motor, operatively connected to a suitable gear wheel (946), typically a worm gear, that is meshable with the said actuating portion (860), so that when engaged, the actuation means (944) provides rotational motion to the sequencing assembly (800) of the required angular displacement and direction when required. A suitable microprocessor means within the control/measuring means (900) controls operation of the

actuation means (942), (944), and coordinates operation of the same according to any software program that is suitable to the test being performed in the reaction vessel (110).

A slot (960) is provided in the operations chamber (920) for receiving the end portion (660) of the electrode means (600) when the reaction vessel (110) is mounted within the operations chamber (920). The control/measuring means (900) typically includes suitable terminals for providing electrical contact with the contacts (635), (645) and (655), and further comprises suitable circuit means for generating from the electrode potential of the electrode (600) an electric current and measuring its intensity, and digital elaborating means, comprising a memory and a CPU programmed for deriving from said current intensity a signal representing the parameter (e.g. the antigen concentration) that represents the results of the assay. This generally requires storing in said memory a set of comparative or zero data. For instance, if it desired that the control/measuring means (900) should display the concentration of the target substance from the electrode potential and display its value, it is possible to operate the system with a number of solutions having known concentrations of the target substance and to memorize a table corresponding to a curve representing concentration as a function of electrode potential, viz. listing concentration values versus potential values. From such a table, the CPU can calculate the concentration of the target substance in each tested body fluid. A display (980) displays data relating to the operation of the control/measuring means (900), prompts the user to execute an action or provide a command, and displays results of the test carried out.

While electronic devices capable of carrying out the above measurement and control operations of the control/measuring means (900) could be easily designed by skilled persons, many are available on the market in the form of chips.

A preferred example of the use of the reaction vessel of the invention is the determination of TXB_2 or its metabolites or metabolites of TXA_2, that are contained in a patient's urine. However, the system is usable for any other immunoassay, with the provision of the appropriate antibody and optionally enzyme are immobilised on the solid phase support. Such antibodies may be provided from any suitable source, and may include human antibodies, animal antibodies, and artificial antibodies such as for example as may be obtained from molecular imprinting processes. The solid phase support is typically prepared with the antibody and enzyme before the assay.

In one type of immunoassay method, for which the system of the invention is typically used, the solid phase support has covalently immobilised therein GOX or a similar oxidase, capable of generating hydrogen peroxide from a suitable substrate, and an antibody that is capable of specifically binding to the antigen of interest to be assayed. To the sample tested there are added a peroxidase-labeled antibody-antigen conjugate, wherein the antigen is the same as the analyte of interest, as well as the other components required to create a redox system with the aforesaid oxidase, e.g., aminosalicylic acid, and glucose and iodide ions. A typical peroxidase may be HRP, for example. The GOX catalyzes the oxidation of glucose to H_2O_2. Iodine is produced in the peroxidase-catalyzed H_2O_2/iodide redox system. If the antigen of interest is absent from the sample, the antibody immobilised on the solid phase support binds only the peroxidase-labeled antibody-antigen conjugate, and the degree of said reaction is measured amperometrically by the electrochemical reduction of iodine back to iodide. If there was antigen present in the sample, this unconjugated antigen competes with the peroxidase-labeled antibody-antigen conjugate for the available sites of the antibody immobilised on the solid phase support; or, alternatively, displaces the antigen from the conjugate. In these cases, the degree of said reaction is correspondingly lower and the amount of antigen in the tested liquid can be calculated from the reduction of said degree. Said method, however, is mentioned by way of illustration only and does not constitute a limitation to the use of the present invention.

In another mode of operation, peroxidase-labeled antibody may be used in place of the peroxidase-labeled antibody-antigen described above. In this "sandwich" type of assay, the antigen present in the sample is bound by one or more epitopes to the antibody immobilised on the solid phase support and by different epitopes to a peroxidase-labeled antibody. In this mode of operation, the amperometric signal increases with increasing concentrations of antigen in the liquid sample.

In particular, the said solid state support according to the present invention is preferably in the form of suitable beads, of Protein A agarose for example, onto which may be immobilised a specific antibody for a particular immunoassay. Alternatively, the beads may made from acrylamide, methacrylate, polystyrene, agarose or cellulose, for example. The beads are typically coated with any suitable amines, carboxylates, aldehydes, carbonyls, hydroxyls, hydrazides or nitrites, or, protein A, B or G. The beads may be provided within the reaction chamber (340) itself, supported over the screen member (345). Alternatively, and preferably, the beads are stored in the upper portion (200) until required for the particular test, and then delivered to the reaction chamber (340). Accordingly, the beads may be stored in any one of the holding chambers (20A), (20B), (20C), (20D) or (20E), typically the first one thereof that is activated to open and deliver its contents to the reaction chamber (340). Preferably, though, the beads are comprised within the lumen (715) of the stirrer assembly (700), which comprises a removable cap (not shown) at the lower end thereof to prevent premature egress of the beads out of the lumen. This is of particular importance in cases where the upper portion (200) is kept separate to the lower portion (300) until shortly before performing a test. When required, the cap at the lower end of the stirring assembly (700) is removed and the beads are forced into the reaction chamber (340) by injecting the sample fluid to be tested via the lumen (715).

The Example at the end of this description includes a typical method for preparing beads, according to the present invention.

Alternatively, the solid support may comprise a suitable oxidised, cellulose paper, as described for example in copending International Application Number PCT/IL00/00650 filed by applicants, the contents of which are incorporated herein in their entirety by reference. The oxidised cellulose paper may line the inner cylindrical wall of the reaction chamber (340) such as to provide direct contact between the paper and the contents of the reaction chamber. Alternatively, the oxidised paper may be mounted onto the electrode means (600). Alternatively, the solid state support may be in the form of a suitable membrane made from cellulose, polyacrylamide, polypropylene, polystyrene or polyethylene, for example.

Alternatively, the solid state support may be in the form of a film or coating of a suitable immobilising substrate applied to the inner cylindrical wall or any other part of the reaction chamber (340) such as to provide direct contact between the coating and the contents of the reaction chamber. Alternatively, the solid state support may be in any other suitable form.

A preferred example of the use of the reaction vessel (110) of the invention is the determination of TXB_2 or its metabolites or metabolites of TXA_2, that are contained in a patient's urine. An important consideration in amperometric immunoassays is that the concentration results obtained therewith are heavily influenced by dilution level of the sample, such as urine sample, i.e., if the patient happens to drink a quantity of water before the urine sample is collected, this would artificially reduce the concentration of the thromboxane measured. Accordingly, the concentration results obtained in any such immunoassay need to be normalised with respect to the urine dilution. Prior art methods for establishing this normalisation are by determining the osmolarity of the urine or the creatinine concentration thereof, which require a

separate test for performing either evaluation, and the subsequent combining of the results with the results obtained by the immunoassay.

According to the present invention, such normalisation is performed with the same apparatus as is being used for the immunoassay, and concurrently therewith, providing normalised thromboxane concentration results in a relatively simple and straightforward manner. In particular, such normalisation is performed by a method incorporating an electrical conductivity value obtained for the urine sample. Such a method is disclosed in co-pending Israel Patent Application Nos. 137307 and 137308 by applicants, filed on 13 July 2000, the contents of which are included herein in their entirety by reference, and essentially involves dividing the value for thromboxane concentration determined in the immunoassay by the value of electrical conductivity of the urine before the immonoassay is performed.

Accordingly, operation of the system (999) starts with the mounting of the reaction vessel (110) within the operations chamber (920) and the subsequent delivery of a sample of urine, typically about 1cc, from the patient to the reaction chamber (340) via opening (716) and lumen (715), which may be accomplished with the aid of a syringe, for example. The urine sample typically comprises about 1,4 ml of filtered urine taken from a patient. Where the lower end (717) is reversibly closed, for example by a removable cap, the force of injection of the sample enables the lower end (717) to be opened, thus providing communication between the reaction chamber (340) and the outside (500). In the preferred embodiment, the beads are originally stored in the lumen (715) and are flushed downwards into the reaction chamber (340) from the lumen (715) by the sample itself. Typically, about 80μm of well-stirred solution containing the prepared beads is provided in the lumen (715). The urine sample is allowed to cool and the beads to settle to the bottom of the reaction chamber (340) for a predetermined dwell time, typically about 30 seconds. Measurement of urine conductivity is then performed by means of the electrode means (600), and the measured average value C_1 after a few seconds

is stored by the electronic control/measurement means (900). The reaction vessel (110), having all the necessary chemicals already contained in holding chambers (20A) to (20E) in a known or predetermined order of use for the particular test being performed, is then mounted in the operations chamber (920), engaging the electrode means (600) into the slot (960) provided therefor. The sequencing portion (850) is set with respect to the plugging assembly such that the cam element (855) assumes a neutral position inbetween the third holding chamber (20C) and the fourth holding chamber (20D). According to commands from the control/measuring means (900), the actuation means (944) rotates the sequencing assembly (800) through the required arc such as to bring the cam element (855) into opening position for the fourth plugging member (20D), opening the corresponding upper opening (22D) for a pre-set period, typically between about 10 to 20 seconds, and thus allowing the contents therein, typically about 700μl of 0.385μg/ml HRP-Tx in a wash/binding buffer which contains for example 2% BSA and 0.01% Thimerosal, to flow into the reaction chamber (340) via gutter (350D) and lumen (310). By way of non-limiting example, HRP-Tx stock solution may be made by mixing 50 ml of a buffer solution (made from 20 ml of 5% BSA, 29.5 ml 0.1M Bind and Wash solution and 0.5 ml of 1% Thimerosal) with 325 μl of a HRP-Tx 50μm/ml solution (wherein 1 ml of this solution is made with 1 ml of urine dilution buffer solution (see below) and 20 μl of HRP-Tx 2.5 mg/ml). Then, the actuation means (944) rotates the sequencing assembly (800) through the required arc such as to bring the cam element (855) into opening position for the fifth plugging member (20E), opening the corresponding upper opening (22E) for a pre-set period, typically also between about 10 to 20 seconds, and thus allowing the contents therein, typically about 500 μl of urine dilution buffer solution, to flow into the reaction chamber (340) via gutter (350E) and lumen (310). By way of non-limiting example, 100 ml of urine dilution buffer solution is made from 89 ml 0.1M Bind and Wash solution (pH 7.4), 10 ml of 1% BSA and 1 ml of 1% Thimerosal. Next, the control/measuring means (900) commands the other actuation means (942) to stir the contents of the reaction chamber (340) for a predetermined period, for example about 10

minutes, via said stirring assembly (700). This is known as the competition stage. After stirring stops, the actuation means (944) rotates the sequencing assembly in the appropriate direction and over the required angular displacement until plugging element (272) is opened, enabling the liquid contents of the reaction chamber to be drained into the waste chamber (360). Stirring is re-initiated for a pre-set period, typically about 20 seconds, to maximise expulsion of the liquid contents to the waste chamber (360).

The actuation means (944) then advances the cam in the original direction to enable holding chamber (20A) to deliver about 3 ml - typically - of a first washing solution contained therein to the reaction chamber (340), and thus opens the plugging element (26A) for a predetermined period, typically about 10 to 20 seconds. By way of non-limiting example, one litre of 0.1M first washing solution may be made from 84.5 ml of 1M Na_2HPO_4, 15.5 ml of 1M NaH_2PO_4 and 150ml of 1M NaCl, plus 750ml of water. Next, the control/measuring means (900) commands the actuation means (944) to rotate the sequencing assembly in the reverse direction until plugging element (272) is opened, enabling the liquid contents of the reaction chamber (340) to be drained into the waste chamber (360). Stirring is again re-initiated for a pre-set period, typically about 20 seconds, to maximise expulsion of the liquid contents to the waste chamber (360).

The actuation means (944) then advances the cam in such as to enable holding chamber (20B) to deliver about 3 ml -typically - of a second washing solution contained therein to the reaction chamber (340), and thus opens the plugging element (26B) for about 20 seconds. By way of non-limiting example, one litre of 0.025M second washing solution may be made from 250ml of the first washing solution as exemplified above, together with a further 112.5 ml of 1M NaCl, plus 637.5ml of water. At this stage, a second measurement of conductivity is performed and the measured average value C_0 after a few seconds is stored by the electronic control/measurement means (900). This measurement checks the proper functioning of the control/measurement

means (900), and also of the electrode means (600), since the value of C_0 that should be obtained at this stage is generally known.

Then, the current in the reaction chamber (340) is measured via the electrode means (600) and the measured average value I_0 after a few seconds is stored by the electronic control/measurement means (900). This measurement initialises the electrode function.

Next, the control/measuring means (900) commands the actuation means (944) to rotate the sequencing assembly in the reverse direction until plugging element (272) is opened, enabling the liquid contents of the reaction chamber (340) to be drained into the waste chamber (360). Stirring is again re-initiated for a pre-set period, typically about 20 seconds, to maximise expulsion of the liquid contents to the waste chamber (360).

In the next stage, actuation means (944) advances the cam (855) in the original direction to enable holding chamber (20C) to deliver about 2 ml - typically - of a detection solution contained therein to the reaction chamber (340), and thus opens the plugging element (26C) for about 20 seconds. By way of non-limiting example, one litre of 0.1M detection solution may be made from 3.95 ml of 1M Na_2HPO_4, 46.05 ml of 1M NaH_2PO_4 and 16.66 ml of 3M KCl, plus 433.34 ml of water, having a pH of 5.9 To this buffer solution, 500 ml of TMB solution (Sigma cat. No. T8665) is added. Stirring is re-initiated and the current in the reaction chamber (340) is measured. In this detection stage, after about 15 seconds of measuring an initial value I_1 of current, averaged over about 5 seconds is stored by the electronic control/measurement means (900). After about 75 seconds from the onset of measuring, a final value I_2 of current, averaged over about 5 seconds is stored by the electronic control/measurement means (900).

Typically, the electronic control/measurement means (900) requires the user to input thereto whether or not the patient who supplied the urine sample has

taken aspirin or not. The electronic control/measurement means (900) then performs thromboxane concentration calculations based on the value of I_1 and I_2 measured, as well as on the conductivity of the urine sample based on the value of C_1, followed by calculation of normalised thromboxane concentration, also taking into account whether or not aspirin was taken. Typically, software in the electronic control/measurement means (900) converts the difference (I_1-I_2) into a TxB_2 concentration according to known correlations therebetween, which are typically stored in a suitable memory. Then, the software divides the resulting concentration value by the conductivity value of the sample, C_1, to provide the normalised concentration value of the TxB_2 in the sample tested. Any one of the these parameters, and preferably all, are displayed via the display means (940).

In an assay of a urine sample conducted using the chemicals as described in the preceding paragraphs relating to operation of the system (999), the following results were obtained using the system (999):- Thromboxane concentration (TxB) ~ 805 pg/ml; urine conductivity (Con) ~ 6.11mS; normalised Thromboxane concentration (NTxB) ~ 131.8 pg/ml.mS.

Preferably, the electronic control/measurement means (900) is also able to check for errors and advise the user with appropriate messages. Such errors may include the following:-

- A value of $35 > C_0 > 120$ indicates that measured conductivity is off the scale.
- A value of $35 > C_1 > 120$ indicates that measured urine conductivity is off the scale.
- A value of $-30 > I_0 > 5$ indicates that test current value is off the scale.
- A value of $-50 > I_1$ indicates that initial current value is off the scale.

- A value of $-500 > I_2$ indicates that final current value is off the scale.
- A value of $I_1 = I_2$ indicates an error in measurement.

The following examples illustrate, but do not limit operation of the system (999) according to the present invention.

Example

Thromboxane B_2 - competitive assay on beads

General

The aim of this assay is determine the concentration of TxB_2 in human urine. The assay is based on the competition between TxB_2 and a fixed quantity of HRP-TxB_2 for a limited number of binding sites on anti-TxB_2 antibody. The amount of HRP-TxB_2 bound by the antibody will be inversely proportional to the concentration of tested TxB_2.

Calculation

One ml of serum (α-TxB_2 serum H492) contains 10 mg of IgG, from which 0.05% are the specific antibody.

200 µl of Protein A are needed for 20 µl of serum (0.2 mg IgG, 0.1 µg specific antibody). The concentration of the specific antibody is thus 0.5 ng/µl beads.

Procedure

Materials

Wash/binding buffer: 0.1M Sodium phosphate, 0.15M NaCl pH 7.4 (150 ml of 1M NaCl, 84.5 ml of 1M Na_2HPO_4 and 15.5 ml of 1M NAH_2PO_4 are added to a total volume of 1 liter of DDW).

Working solution: 0.1M phosphate-citrate buffer, pH 5.3, 0.1MCl$^-$, amM ASA. (1 tablet of phosphate-citrate buffer, pH 5, (Sigma cat No. P4809), 3.3 ml of 3M

KCl, 13.5 ml of well shaken 14.8M 5-aminosalicylic acid (one tablet of aminosalicylic acid (Sigma cat No. A6178) in 50 ml of DDW).

Preparation of Beads

200 μl of Protein A (protein A agarose KPL Cat. No. 223-50-00) were washed on a polypropylene column with 3 ml of the wash/binding buffer. The beads were transferred to a 1.5 ml tube with a total volume of 1 ml of the washing/binding buffer and 20 μl of serum were added in one portion. The tube was shaken lightly for one minute and the mixture was replaced onto the column and washed with 6 ml of the wash/binding buffer. After the washing was completed, the mixture was transferred to a tube with a total volume of 2 ml 1:4 wash/binding buffer which contain 0.1% Ovabumin and 0.01% Thimerosal and left to stand over 72 hours at 4°C before use.

Competition procedure

Sample preparation for 3 identical measurements:

A solution of 1.445 ml of the wash/binding buffer, 170 μl of the wash/binding buffer with 1% BSA, 85 μl of the sample and 17 μl of HRP-TxB_2 (10 μg/ml) was prepared in a 2 ml tube.

30 μl of the well-stirred solution of the prepared beads (3 μl beads) was placed on a bio spin column (BIO RAD Cat. No. 732-6008).

The competition assay starts when 0.5 ml of the prepared sample is added to the beads loaded on the column. The columns are shaken in a spatial apparatus for 10 minutes and the mixture is then washed out with 5 x 1 ml wash/binding buffer. At this stage the beads are ready for the electrical measurements.

Electrochemical measurements:

The beads from the column are transferred to a conical vial equipped with a conical stirrer, with a total volume of 2.2 ml of working solution. After the

electrode was introduced into the solution and current is stable, 15.5µl of 0.3M H_2O_2 are added and the measurement begins.

While in the foregoing description describes in detail only a few specific embodiments of the invention, it will be understood by those skilled in the art that the invention is not limited thereto and that other variations in form and details may be possible without departing from the scope and spirit of the invention herein disclosed or exceeding the scope of the claims.

Claims:

1. A reaction vessel for carrying out at least one step of a chemical process and for enabling at least one parameter correlated thereto to be measured, comprising:-

at least one upper holding chamber for containing one or more chemicals needed for said process, each said holding chamber having a first lower outlet means, and further having an upper inlet means for enabling fluid communication with an outside of said reaction vessel;

first plugging means for selectively opening and sealing said upper inlet means;

a reaction chamber for carrying out said process, said reaction chamber being in fluid communication with said at least one holding chamber via said first lower outlet means, said reaction chamber having at least one vent for enabling fluid communication with an outside of said reaction vessel, said reaction chamber further comprising monitoring means for enabling said at least one parameter to be measured;

a lower waste chamber in fluid communication with said reaction chamber via a screen element, said waste chamber having an upper second outlet means for providing fluid communication with an outside of said reaction vessel;

second plugging means for selectively opening and sealing second outlet means;

wherein said first outlet means is adapted to selectively prevent and allow liquid communication therethrough according to whether said upper inlet means is sealed or open, respectively; and wherein said screen member is adapted to selectively prevent and allow liquid communication therethrough according to whether said second outlet means is sealed or open, respectively.

2. A reaction vessel as claimed in claim 1, wherein said first outlet means is adapted to selectively prevent and allow liquid communication therethrough according to whether said upper inlet means is sealed or open by comprising a cross-sectional flow area of magnitude below a predetermined first value.

3. A reaction vessel as claimed in claim 2, wherein said predetermined first value is about 0.8 square mm.

4. A reaction vessel as claimed in claim 1, wherein said screen member is adapted to selectively prevent and allow liquid communication therethrough according to whether said second outlet means is sealed or open, by comprising a net-like structure having an open area/total area ratio below a predetermined second value.

5. A reaction vessel as claimed in claim 4, wherein said second value is about 1%.

6. A reaction vessel as claimed in any one of claims 1, wherein said chemical process comprises an amperometric immunoassay and said at least one parameter comprises an electrical current measurement indicative of a concentration of a predetermined target antigen in a test solution on which said process is carried out.

7. A reaction vessel as claimed in claim 6, wherein said monitoring means comprises suitable electrode means in communication with said reaction chamber.

8. A reaction vessel as claimed in claim 7, wherein said reaction chamber comprises a lateral portal, said electrode means being mounted externally to said reaction chamber and abutting said portal such as to provide communication between said electrode means and the said reaction chamber.

9. A reaction vessel as claimed in claim 8, wherein said electrode means comprises a suitable first anode and a suitable cathode in communication with said reaction chamber, said first anode and a cathode each having electrical connectors that are electrically connectible with respect to an external

monitoring apparatus, said first anode and said cathode being adapted for measuring a current of said test solution when in contact therewith.

10. A reaction vessel as claimed in claim 9, wherein said electrode means is further adapted to provide a measurement of conductivity of a suitable liquid accommodated in said reaction chamber and in communication therewith.

11. A reaction vessel as claimed in claim 10, wherein said electrode means further comprises a second anode, said second anode having an electrical connector that is electrically connectible with respect to an external monitoring apparatus, said second anode and said cathode being adapted for measuring a conductivity of said test solution when in contact therewith.

12. A reaction vessel as claimed in claim 11, wherein said reaction vessel comprises a plurality of said upper holding chambers, each said holding chamber having a corresponding said first lower outlet means in communication with said reaction chamber.

13. A reaction vessel as claimed in claim 12, wherein said reaction vessel comprises between 3 and 7 said holding chambers, and preferably 5 said holding chambers.

14. A reaction vessel as claimed in claim 12, wherein said plurality of holding chambers are arranged circumferentially about a longitudinal axis of said reaction vessel.

15. A reaction vessel as claimed in claim 14, further comprising an upper lumen coaxial with said longitudinal axis and extending from an uppermost part of said reaction vessel to said reaction chamber.

16. A reaction vessel as claimed in claim 12, wherein said corresponding upper inlet means of said plurality of said holding chambers are substantially coplanar and substantially equally displaced from said longitudinal axis of said reaction vessel in a radial direction.

17. A reaction vessel as claimed in claim 16, wherein said second outlet means is substantially coplanar with said upper inlet means, and is

substantially equally displaced from said longitudinal axis of said reaction vessel in a radial direction as said upper inlet means.

18. A reaction vessel as claimed in claim 16, wherein each said corresponding first plugging means comprises a resilient tongue element radially disposed with respect to said longitudinal axis and cantilevered at an outer radial end thereof with respect to a common supporting ring, said tongue element comprising a tab at an inner radial free end thereof, and a stopper at a lower face of said tongue adapted for closing or opening the corresponding said upper inlet means when the corresponding free end of said tongue element is pressed thereagainst or distanced therefrom, respectively.

19. A reaction vessel as claimed in claim 18, wherein said second plugging means comprises a resilient tongue element radially disposed with respect to said longitudinal axis and cantilevered at an outer radial end thereof with respect to said common supporting ring, said tongue element comprising a tab at an inner radial free end thereof, and a stopper at a lower face of said tongue adapted for closing or opening the said second outlet means when the corresponding free end of said tongue element is pressed thereagainst or distanced therefrom, respectively.

20. A reaction vessel as claimed in claim 19 wherein said tabs of said first plugging means and said tab of said second plugging means are each displaced radially from said longitudinal axis by a substantially equal first radial displacement.

21. A reaction vessel as claimed in claim 20, further comprising a common supporting ring, wherein each said first plugging means and said second plugging means are joined at their outer radial ends to said supporting ring.

22. A reaction vessel as claimed in claim 21, wherein said supporting ring is integrally joined to said first plugging means and to said second plugging means.

23. A reaction vessel as claimed in claim 22, wherein said supporting ring, each said first plugging means and said second plugging means is made from silicone.

24. A reaction vessel as claimed in claim 22, wherein said supporting ring, and said tongue element of each said first plugging means and said second plugging means is made from a suitable metal including stainless steel, and wherein each said stopper of each said first plugging means and said second plugging means is made from a silicone.

25. A reaction vessel as claimed in claim 22, further comprising suitable sequencing means for selectively opening or closing each said first plugging means and said second plugging means in response to a predetermined angular rotation of said sequencing means.

26. A reaction vessel as claimed in claim 25, wherein said sequencing means comprises an assembly of a sequencing portion joined for rotation to a first actuating portion and rotatably mounted with respect to said reaction vessel coaxially with said longitudinal axis, said sequencing portion being adapted for selectively opening or closing any one of said first plugging means and said second plugging means in response to a predetermined angular rotation of said first actuating portion.

27. A reaction vessel as claimed in claim 26, wherein said sequencing portion comprises a cam element disposed at a circumferential location with respect to said longitudinal axis, such as to raise a said tab when said cam is angularly aligned with respect to the tab such as to open a corresponding said upper inlet means or said second outlet means.

28. A reaction vessel as claimed in claim 27, wherein said resilient tongues are biased to close the corresponding said upper inlet means or said second outlet means when the corresponding tab is not circumferentially aligned with said cam element.

29. A reaction vessel as claimed in claim 27, wherein said sequencing portion further comprises an upper annular lip adapted for pushing

downwardly any said tab that is not circumferentially aligned with said cam element such as to close the corresponding said upper inlet means or said second outlet means.

30. A reaction vessel as claimed in claim 27, wherein said sequencing assembly comprises a central aperture extending therethrough longitudinally.

31. A reaction vessel as claimed in claim 30, further comprising a stirrer assembly having a shaft rotatably mounted with respect to said central lumen and said central aperture, said shaft having a stirring portion at one end thereof extending into said reaction chamber, and a second actuating portion at the other end thereof extending outside of said reaction vessel.

32. A reaction vessel as claimed in claim 1, wherein said reaction chamber and said waste chamber are comprised in a lower portion, and said holding chambers, said sequencing means, said stirrer assembly, said first plugging means and said second plugging means are comprised in an upper portion, and wherein said upper portion is selectively mountable with respect to said lower portion.

33. A reaction vessel as claimed in any one of claims 1 to 33, wherein said reaction vessel is substantially disposable.

34. A reaction vessel as claimed in claim 33, wherein at least a suitable antibody appropriate for said immunoassay is immobilised in a solid state support provided in said reaction vessel.

35. A reaction vessel as claimed in claim 34, wherein said antibody is provided from any suitable source, and may include at least one of human antibodies, animal antibodies, and artificial antibodies such as for example as may be obtained from molecular imprinting processes.

36. A reaction vessel as claimed in claim 34, wherein said solid state support comprises a plurality of beads coated with a suitable protein.

37. A reaction vessel as claimed in claim 36, wherein said beads are provided in the said reaction chamber prior to commencing said immunoassay.

38. A reaction vessel as claimed in claim 36, wherein said beads are provided in one of said plurality of holding chambers, and wherein said beads may be delivered therefrom to said reaction chamber operative to a predetermined actuation of said sequencing assembly.

39. A reaction vessel as claimed in claim 36, wherein said beads are provided in said central lumen, and wherein said beads may be delivered therefrom to said reaction chamber operative to said test solution being delivered to said reaction chamber via said central lumen.

40. A reaction vessel as claimed in claim 34, wherein said solid state support comprises a layer of a suitable protein provided in an inner wall of said reaction chamber.

41. A reaction vessel as claimed in claim 34, wherein said solid state support comprises a suitable oxidised cellulose paper in said reaction chamber.

42. A system for carrying out an amperometric immunoassay on a test solution having a target antigen, comprising:-

a reaction vessel as claimed in any one of claims 34 to 41, selectively mountable and dismountable with respect to;

suitable electronic control and measuring means for controlling operation of said reaction vessel and for monitoring the response of the electrode means.

43. A system as claimed in claim 42, wherein said control and measuring means are comprised in a suitable housing, said housing further comprising an operations chamber adapted for accommodating therein said reaction vessel during operation thereof.

44. A system as claimed in claim 43, comprising suitable first and second actuation means engageable for rotation with said first actuation portion and second actuation portion, respectively, when said reaction vessel is mounted to said control and measuring means.

45. A system as claimed in claim 43, wherein said control and measuring means is in operative electrical contact with said electrode means to enable

electrical current and conductivity measurements of a test solution accommodated in said reaction chamber to be performed.

46. A system as claimed in claim 45, wherein said electrode means comprises an outwardly extending tip with respect to said reaction vessel.

47. A system as claimed in claim 46, wherein said control and measuring means comprises a terminal operatively connected thereto and adapted for engaging with said tip such as to establish electrical communication therewith when said reaction vessel is mounted to said control and measuring means.

48. A system as claimed in claim 47, wherein said control and measuring means comprises suitable amperometric circuit means for measuring an electric potential of the said electrode means.

49. A system as claimed in claim 48, wherein said control and measuring means further comprises means for digitally processing a change in said electric potential generated in response to said reaction to provide a first signal representative of the immunoassay.

50. A system as claimed in claim 48, wherein said control and measuring means further comprises means for digitally processing a change in said electric potential generated in response to said reaction to provide a second signal representative of the conductivity of the test solution.

51. A system as claimed in claim 50, further comprising suitable memory means for storing comparison values used for enabling calculation of a desired parameter representing the result of the assay from at least one of said first signal and said second signal, and processing means for carrying out such calculation.

52. A system as claimed in claim 51, wherein said parameter is target concentration data.

53. A system as claimed in claim 51, wherein said parameter is normalised target concentration data.

54. A system as claimed in claim 51, further comprising a suitable electronic display adapted for displaying said parameter.

55. A system as claimed in claim 54, wherein said display is comprised in the said control and measuring means.

56. A system as claimed in claim 42, wherein each said upper holding chambers comprises suitable chemicals required for the particular immunoassay being performed.

57. A system as claimed in claim 42, wherein one said holding chamber comprises an oxidase-labeled antibody-antigen conjugate, wherein the antigen is the same as the analyte of interest, and components required to create a redox system with the aforesaid oxidase.

58. A system as claimed in claim 42, wherein said oxidase-labeled antibody-antigen conjugate is horseradish peroxidase-labeled antibody-antigen conjugate.

59. A system as claimed in claim 42, further comprising suitable portable power means.

60. A system as claimed in claim 59, wherein said power means comprises at least one electrical battery.

61. A system as claimed in claim 42, wherein the antibody immobilised in the solid substrate is that required for the determination of TXB_2 or any one of the metabolites thereof, or any one of the metabolites of TXA_2.

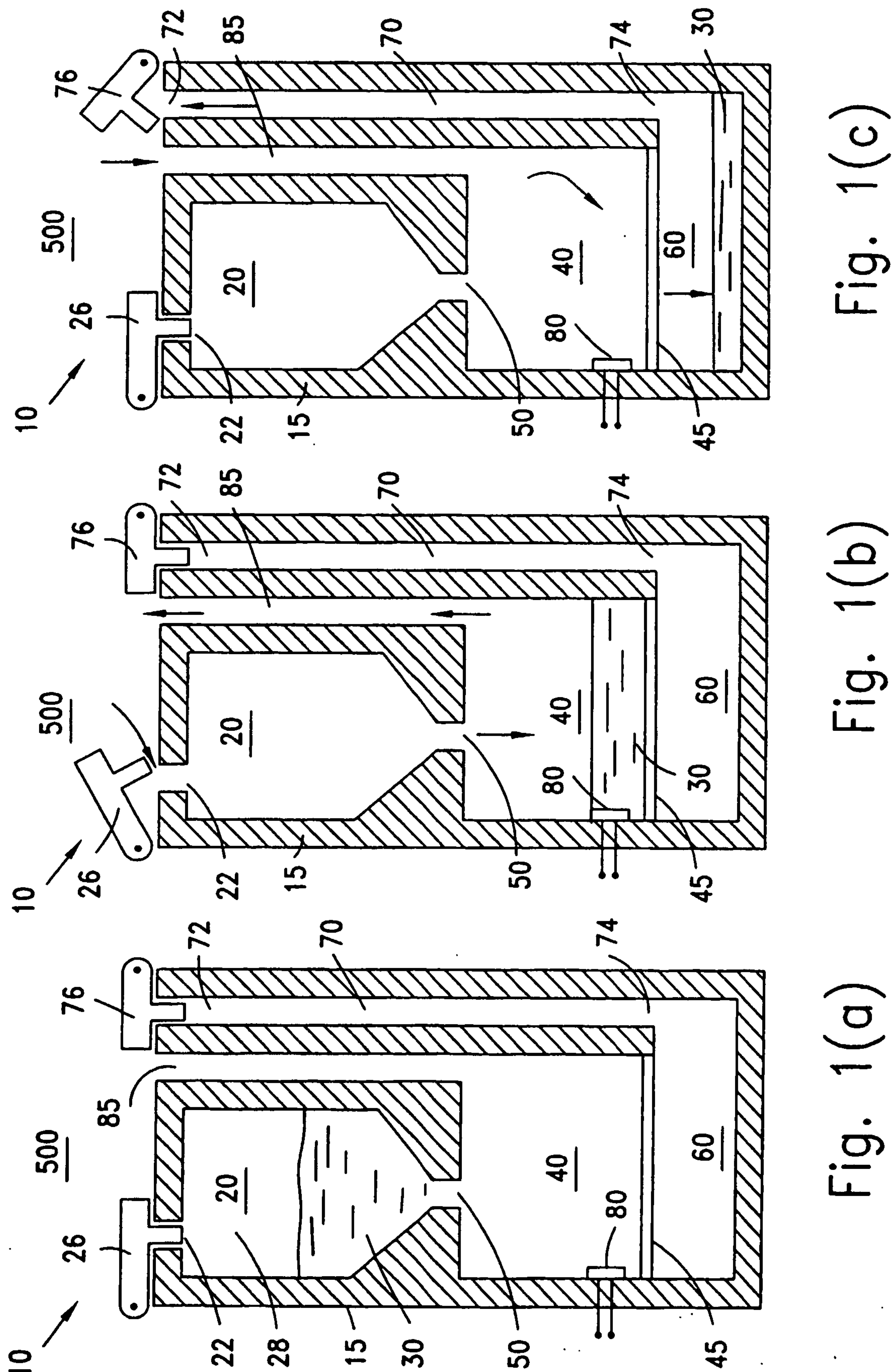
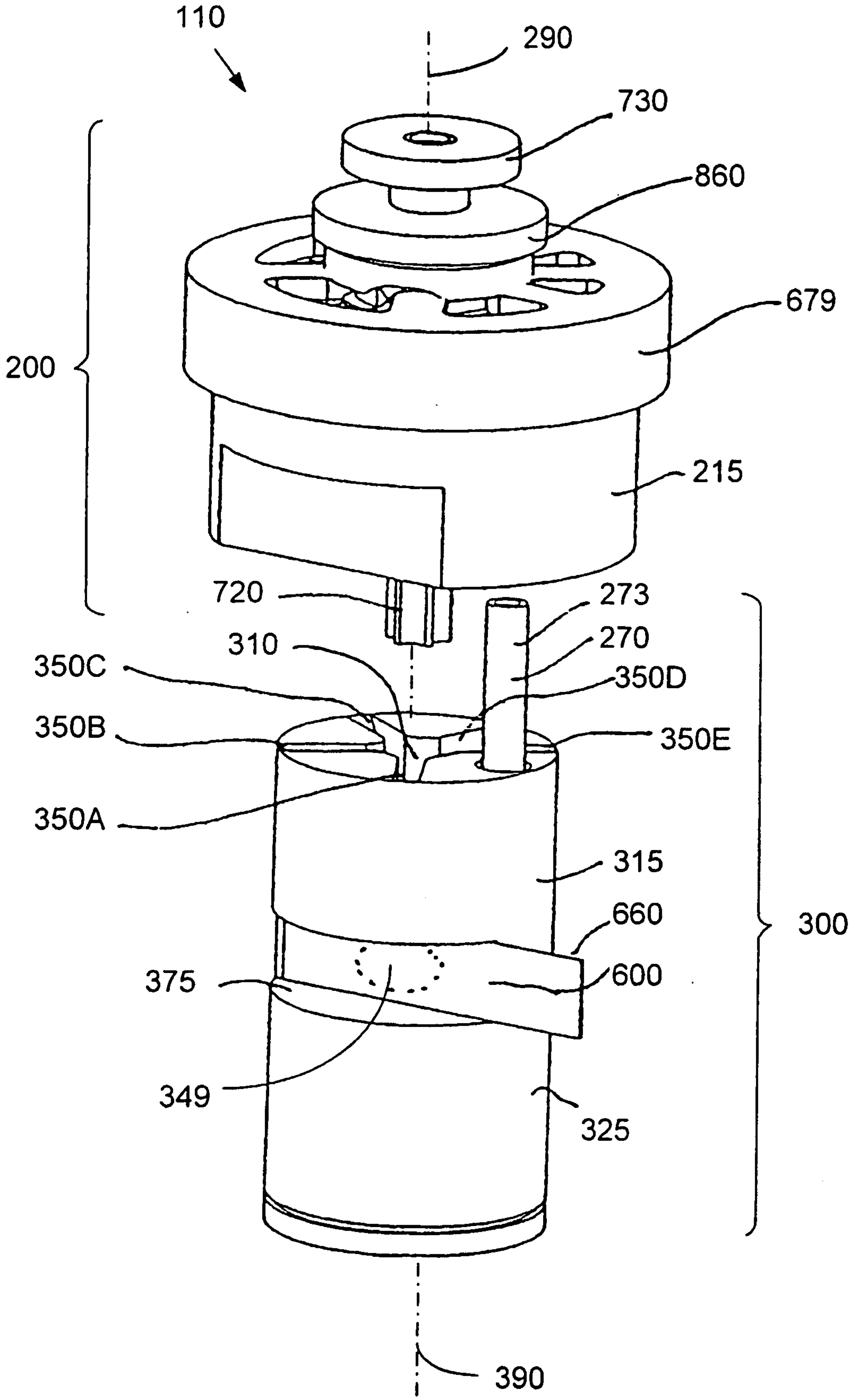


Fig. 1(a)

Fig. 1(b)

Fig. 1(c)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679
200
215
720
273
270
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350C
350D
350B
350E
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315
660
300
375
600
349
325
390

Fig. 2

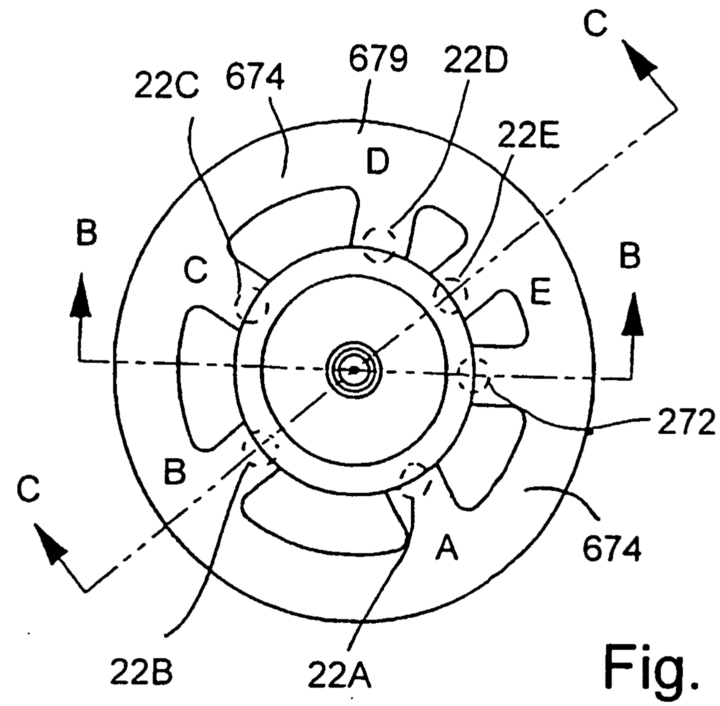
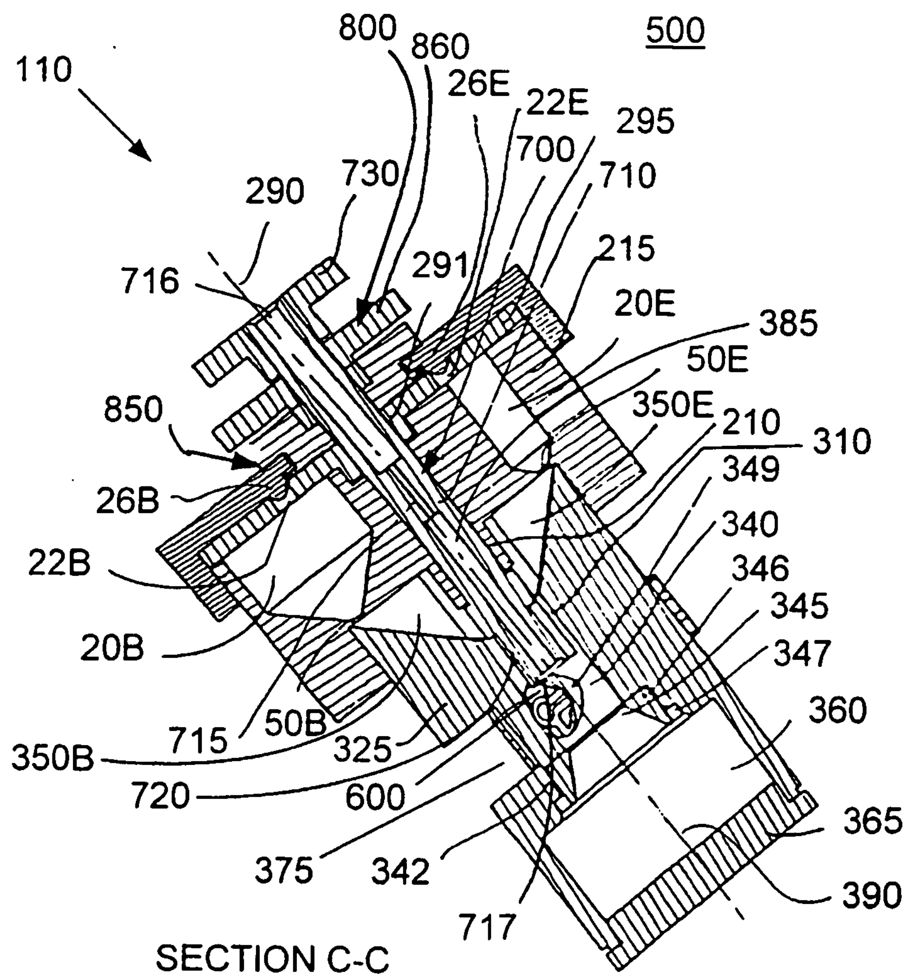
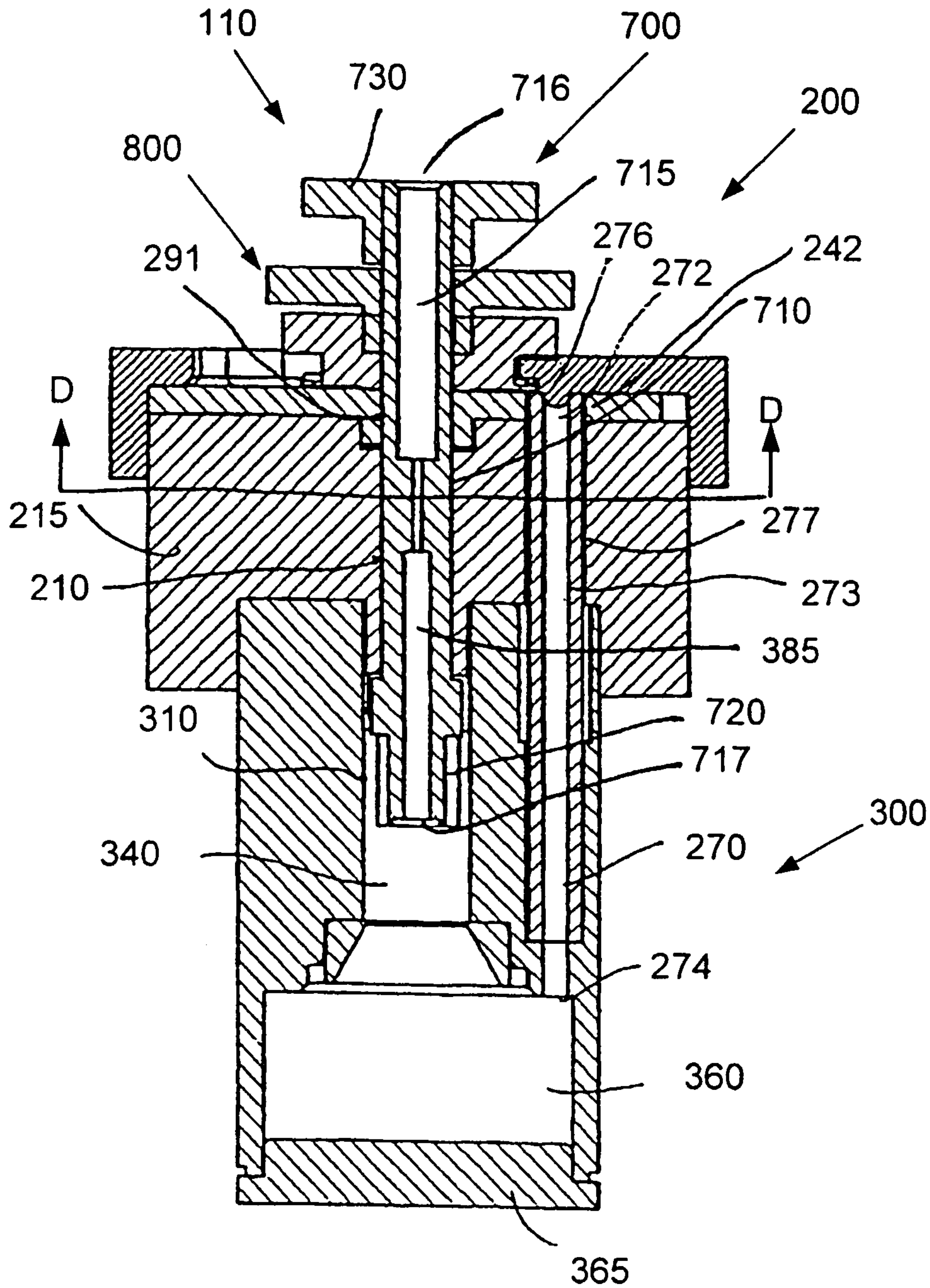


Fig. 3



SECTION C-C

Fig. 4



SECTION B-B

Fig. 5

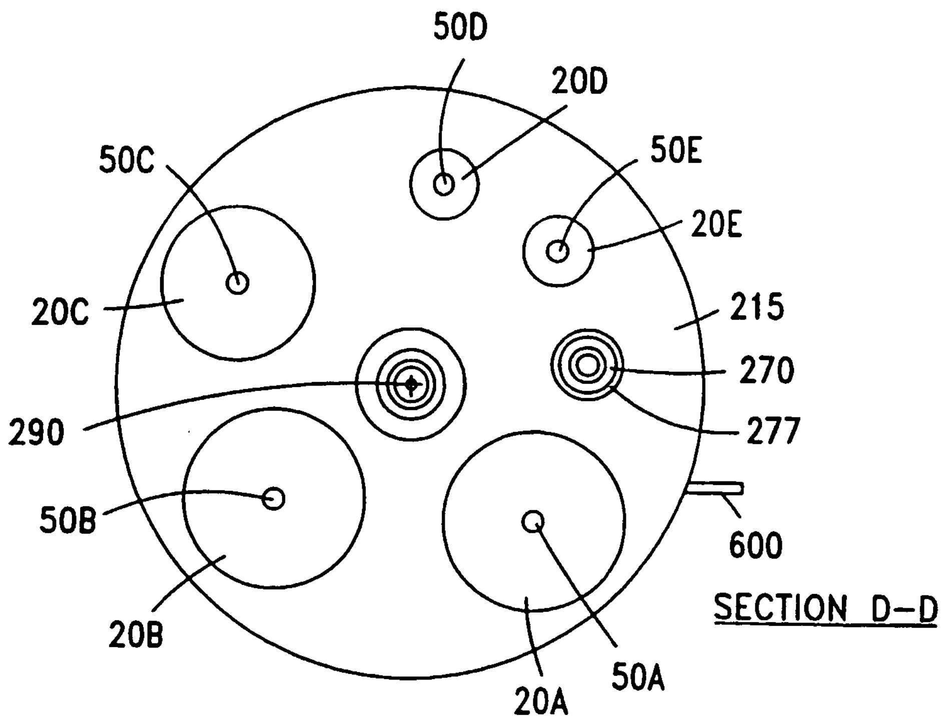


Fig. 6

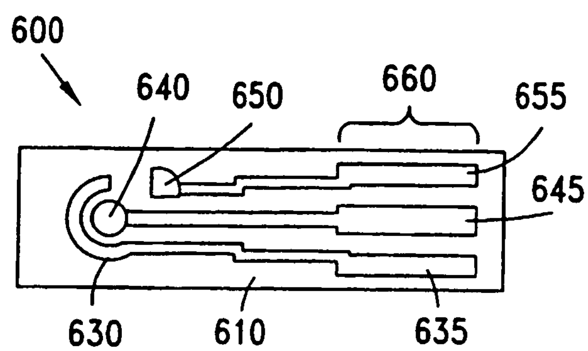


Fig. 7

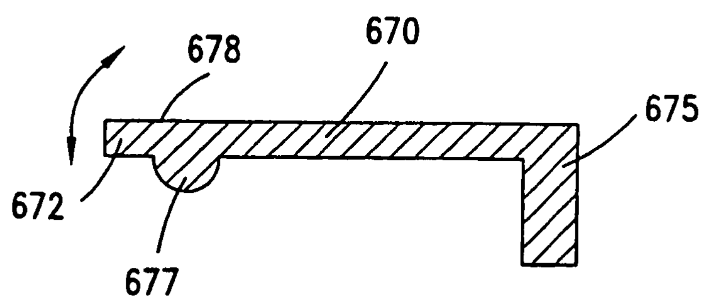


Fig. 8

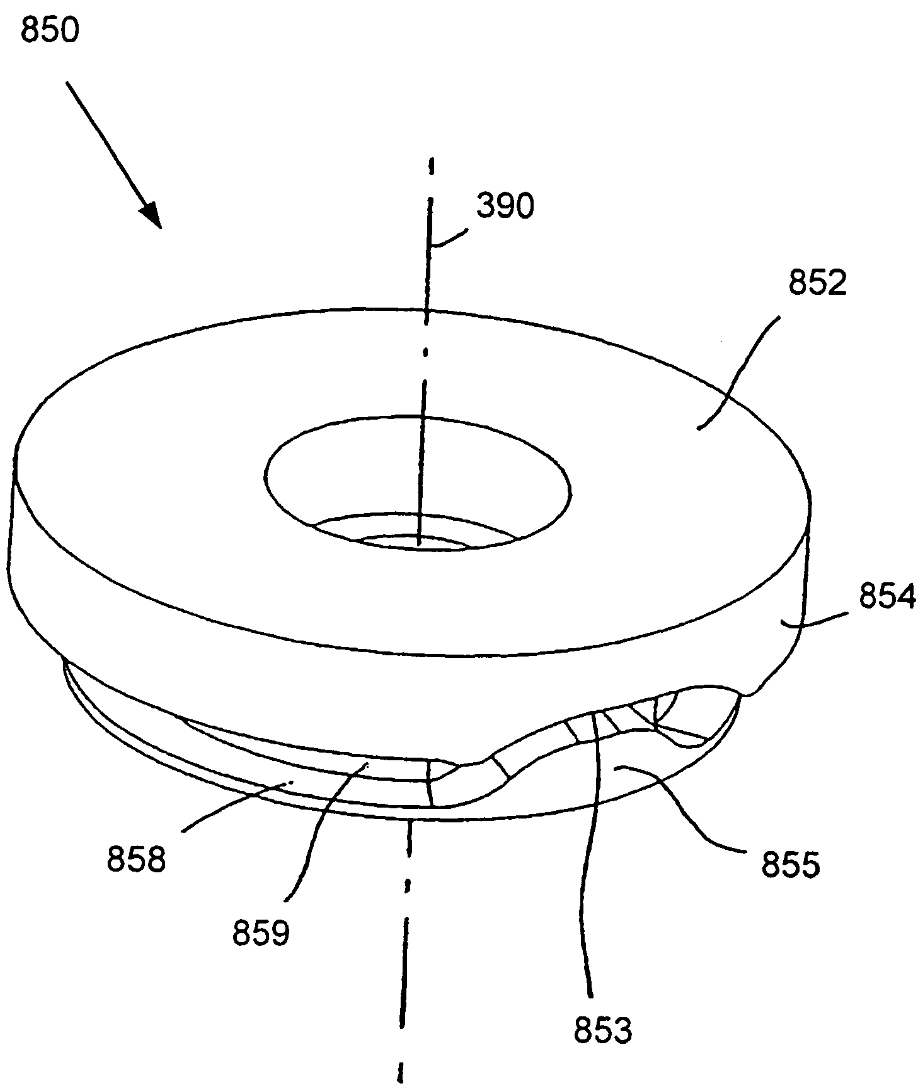


Fig. 9

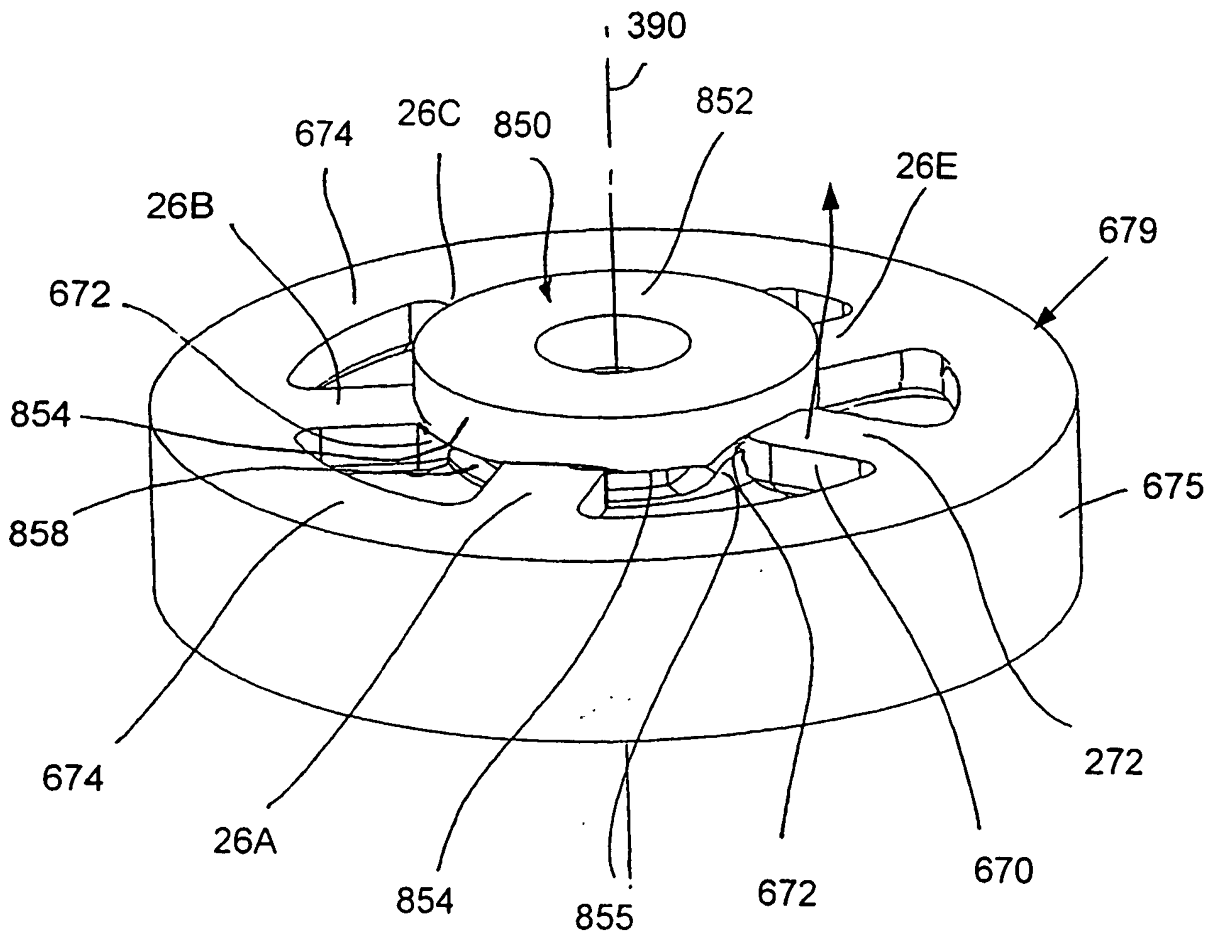

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Fig. 10

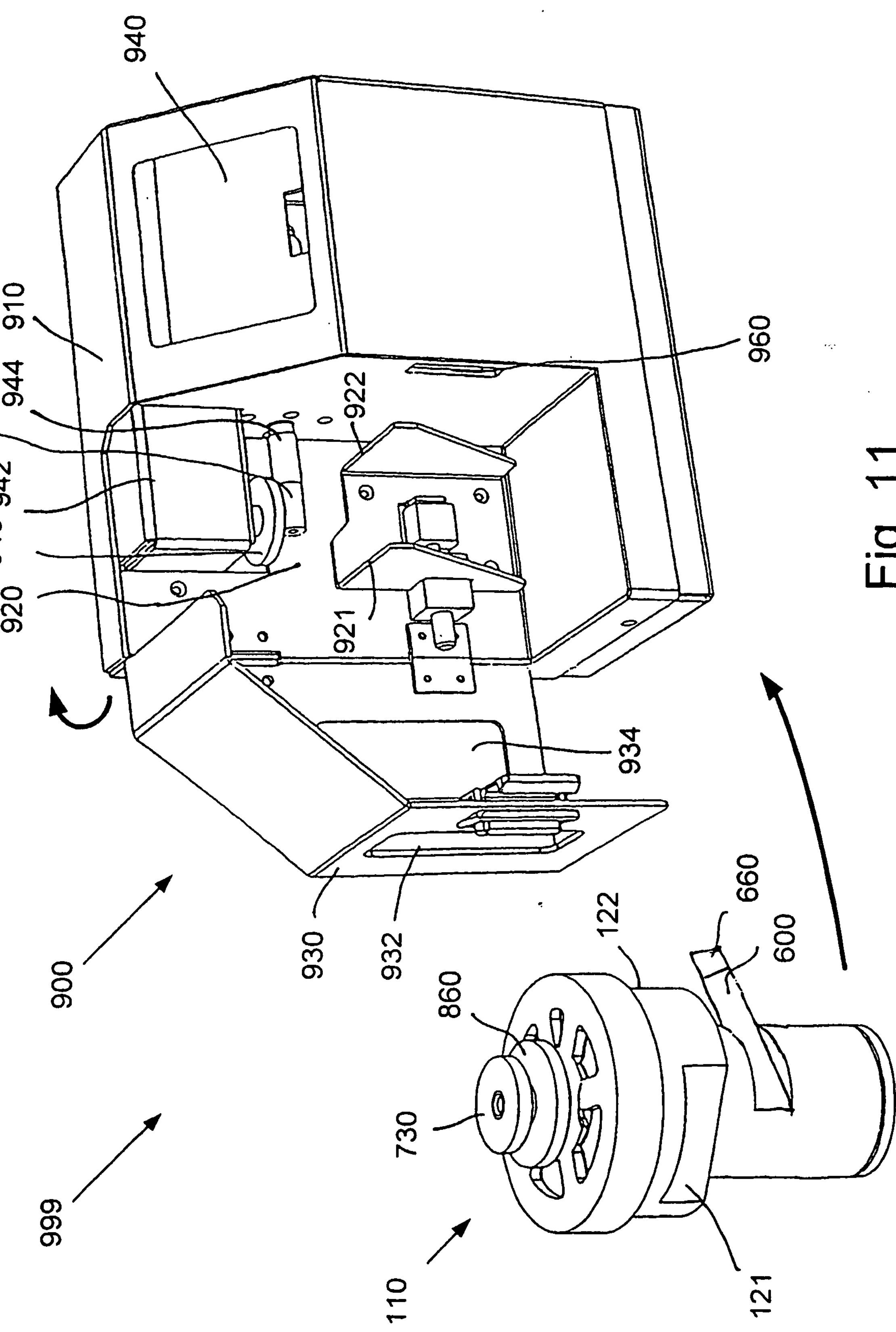
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Fig. 11

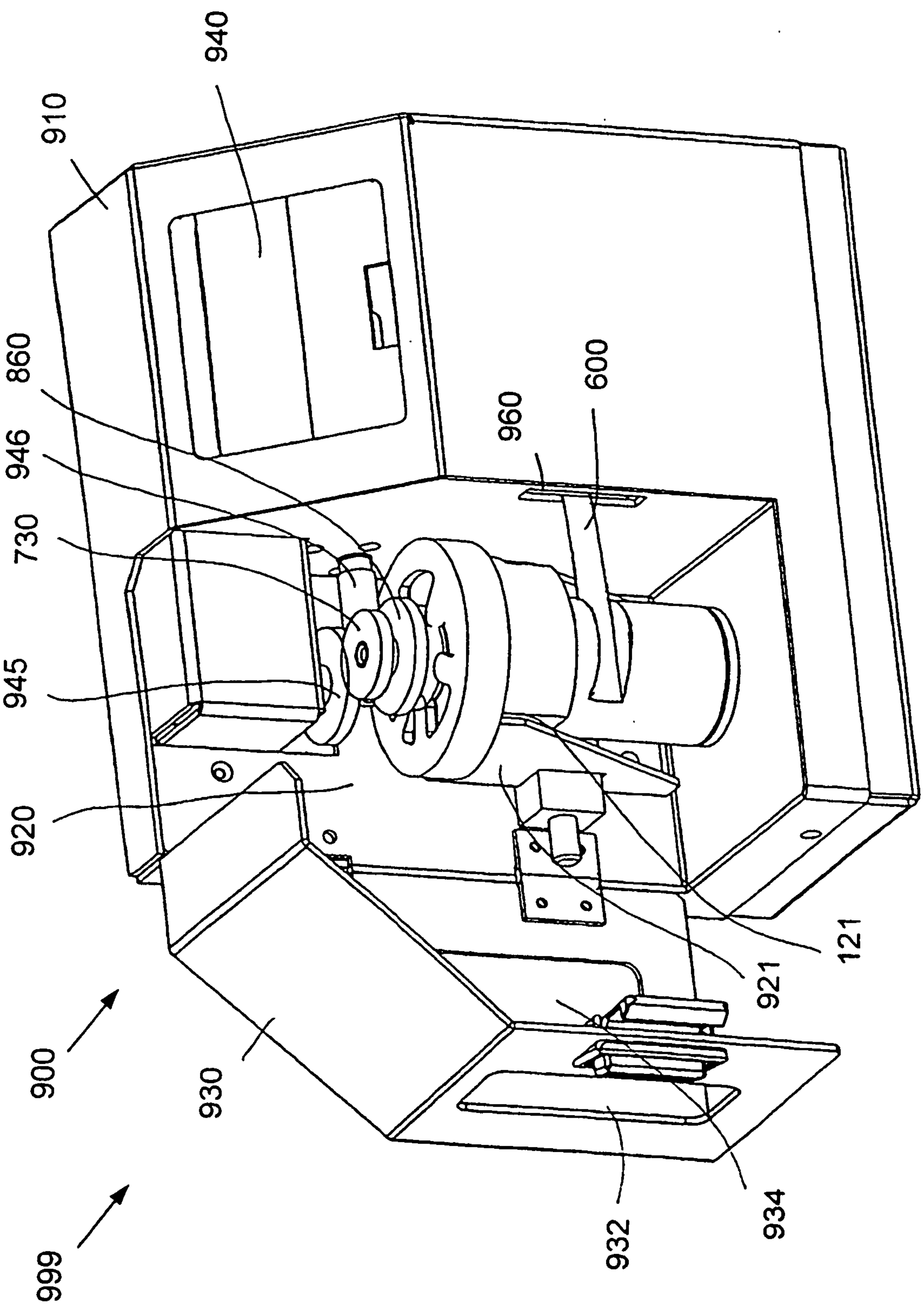

Fig. 12

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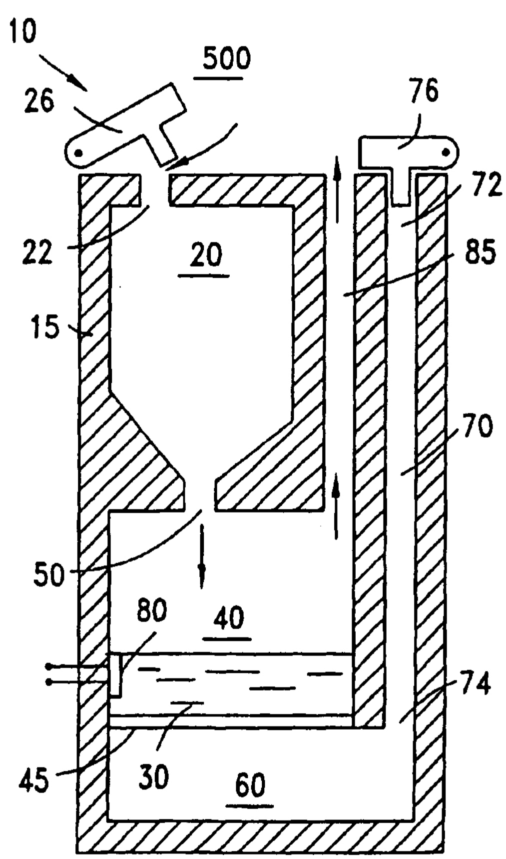
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: REACTION VESSEL AND SYSTEM INCORPORATING SAME



(57) Abstract: A reaction vessel for carrying out at a chemical process and for enabling measurement of parameters correlated to the process, and a system incorporating the vessel. The vessel, which is preferably disposable, has a plurality of upper holding chambers for containing chemicals needed for the process, each holding chamber having a lower outlet and an upper inlet. A plug selectively opens and seals the upper inlet of each chamber. The outlets enable the chemicals to flow into a reaction chamber in a desired sequence for carrying out said process only when the corresponding upper inlet is open. The reaction chamber has a vent with respect to the outside of the vessel. A monitoring electrode is exposed to the reaction chamber for measuring parameters associated with the chemical process. A lower waste chamber having an upper outlet is separated from the reaction chamber via a screen element, and a second plug selectively opens and seals this outlet. The screen member is characterized in selectively preventing and allowing liquid communication therethrough, according to whether the upper outlet is sealed or open, respectively.

WO 02/058845 A3

Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

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X	WO 99 46045 A (OSTERLOH DIRK KLAUS ;PETERS RALF PETER (DE); UENAL NEZIH (DE); BAC) 16 September 1999 (1999-09-16) page 4, line 1 -page 5, line 31 page 13, line 23 -page 15, line 4 page 29, line 11 -page 29, line 24 ---	1-6, 32-41
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